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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	GSGE-001/01US 29258/14
	Application Number	
Title of Invention	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION	

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Prefix	Given Name	Middle Name	Family Name	Suffix
	Gregory		Porreca	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Cambridge	State/Province	MA	Country of Residence i	US

Citizenship under 37 CFR 1.41(b) i: US

Mailing Address of Applicant:

Address 1	57 Reservoir Street		
Address 2			
City	Cambridge	State/Province	MA
Postal Code	02138	Country i	US

Applicant 2 Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Mark		Umbarger	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Brookline	State/Province	MA	Country of Residence i	US

Citizenship under 37 CFR 1.41(b) i: US

Mailing Address of Applicant:

Address 1	5 Winchester Street		
Address 2	Unit 101		
City	Brookline	State/Province	MA
Postal Code	02446	Country i	US

Applicant 3 Remove

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	George		Church	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Brookline	State/Province	MA	Country of Residence i	US

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Citizenship under 37 CFR 1.41(b) [i]	US			
Mailing Address of Applicant:				
Address 1	218 Kent Street			
Address 2				
City	Brookline	**State/Province**	MA	
Postal Code	02146	**Country**[i]	US	

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Customer Number	21710		
Email Address	ip@brownrudnick.com	Add Email	Remove Email
Email Address	aschoen@brownrudnick.com	Add Email	Remove Email
Email Address	tmeyers@brownrudnick.com		Remove Email

Application Information:

Title of the Invention	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION		
Attorney Docket Number	GSGE-001/01US 29258/14	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		**Sub Class (if any)**	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)	3	**Suggested Figure for Publication (if any)**	3

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Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	non provisional of	61426817	2010-12-23

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If the Assignee is an Organization check here. ☒			
Organization Name	Good Start Genetics, Inc.		
Mailing Address Information:			
Address 1	8 St. Mary's Street		
Address 2	Suite 603		
City	Boston	**State/Province**	MA
Country [i] US		Postal Code	02215
Phone Number		Fax Number	
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Signature	/s/Thomas Meyers/			Date (YYYY-MM-DD)	2011-04-07
First Name	Thomas	Last Name	Meyers	Registration Number	36989

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METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

Related Application

The present application claims the benefit of and priority to U.S. provisional patent application serial number 61/426,817, filed December 23, 2010, the content of which is incorporated by reference herein in its entirety.

Field of the Invention

The invention generally relates to methods for maintaining the integrity and identification of a nucleic acid template in a multiplex sequencing reaction.

Background

Sequencing-by-synthesis involves template-dependent addition of nucleotides to a template/primer duplex. Nucleotide addition is mediated by a polymerase enzyme and added nucleotides may be labeled in order to facilitate their detection. Single molecule sequencing has been used to obtain high-throughput sequence information on individual DNA or RNA. The ability to multiplex samples, i.e., pool different patient samples, is important for decreasing costs and increasing the through-put of sequencing-by-synthesis platforms.

One issue that presents itself in a multiplex sequencing reaction is maintaining accurate identification of a sample throughout the sequencing process. Unfortunately, the sample preparation phase frequently introduces errors into the subsequent sequencing reaction. For example, template molecules may physically overlap on a substrate; and in some cases result in a detectable erroneous signal. In any sequencing process that requires high accuracy, such errors can have a significant impact on results. Next generation sequencing typically involves the generation of an in vitro library; arraying of nucleic acid templates into physically distinct locations on a solid support (and optional clonal amplification of the templates into spatially localized clusters); and the sequencing-by-synthesis reaction itself. Errors can occur during generation of the library, arraying and amplification steps that result in molecular overlap of different patient samples. For example, heteroduplexes, i.e., improper pairing of template strands from different samples, may form during the amplification of the multiplexed samples.

Molecular overlap during any of the first three steps in the process may lead to assigning sequencing data to the wrong patient sample. Other errors can be introduced due to incomplete amplification, stray labeled primers, and other sources that result in erroneous signal detection. In a single molecule sequencing protocol, such errors can have a significant affect on the results. Accordingly, methods for reducing or eliminating errors in such procedures are needed.

Summary

The invention generally provides methods for validating the results of a molecular detection assay and for enabling the detection of errors introduced in sample preparation. The invention is applicable to nucleic acid sequencing, protein detection, and other methods that involve precise measurement of the presence and/or amount of an analyte. The invention utilizes two or more identifiers that are uniquely associated with an analyte of interest, such that the identifiers will only be present when the analyte of interest is detected. In this way, false positive and/or false negative results are avoided by requiring concomitant detection of two or more independent markers associated with the analyte to be detected.

The invention is especially useful in multiplex next-generation sequencing applications in which errors can have a significant impact on results, but is also applicable across a broad range of detection assays, including protein detection assays. The invention is based upon using two or more identifiers, such as nucleic acid barcodes that are uniquely associated with an analyte to be detected. For example, in sequencing applications, a pair of barcodes are placed such that they flank the sequence of interest. The identifiers are unique to the sample, so that valid sequence data are confirmed by the presence of the pair of flanking identifiers. In contrast, template containing only one identifier or an incorrect pair (i.e., a pair or more of identifiers not associated with the same template) will be excluded from analysis. Thus, methods of the invention make it possible to determine whether the integrity of a sample was maintained during a multiplex sequencing reaction, and prevent the assigning of sequence data to the wrong sample.

In other applications, two or more nucleic acid tags are associated with a protein or other analyte of interest. Identification of the sequence tags confirms the accuracy of the detection of the protein. In this embodiment of the invention, a protein is captured by a binding agent, such as an antibody, that is coupled to at least two oligonucleotides of known sequence. The identification of the captured protein is confirmed via sequencing of the tags that are uniquely

associated with the binding agent. The presence of both sequence tags validates the capture of the target protein.

In protein detection, identifiers can also include detectable labels, antibodies, other proteins, including glycoproteins, vitamins, steroids, viral particles, and the like that bind specifically to a protein of interest. Detection of the identifiers validates that the protein of interest has been found.

In nucleic acid sequencing, preferred identifiers are nucleic acid barcodes. The barcodes can be of any appropriate length (e.g., from about 2 to about 50 nucleotides) and any number of barcode sequences can be used. Preferably, the barcodes are present in pairs that flank the template to be sequenced, or portions thereof. While the pair of barcodes associated with any given template is unique, individual barcode sequences can be used with multiple templates as long as no pair is used across two different samples. While it is possible, and may be preferred in some circumstances, to use more than two barcoded sequences or other identifiers, the invention will be exemplified using pairs of identifier molecules. In a preferred configuration, each template from a particular sample is associated with the same unique pair of barcode sequences. The pairs ideally flank the region of the template that is to be sequenced. Thus, the pairs can be contiguous with a region of interest or can be separated from the region of interest by spacers (which include intervening sequence in the template or inserted spacer sequence) or by naturally-occurring sequence. Other identifiers, such as nucleic acid binding proteins, optical labels, nucleotide analogs, and others known in the art can be used as identifiers according to the invention.

In certain aspects, methods of the invention involve obtaining a template nucleic acid, incorporating a pair of sequence identifiers into the template, and sequencing the template. Methods of the invention may further include directly or indirectly attaching the template to a substrate. The template may be amplified prior to attachment to the substrate or may be amplified on the substrate after it has been attached to the substrate. In particular embodiments, the template is amplified after it has been attached to the substrate. In other embodiments, the template is amplified before being attached to the substrate and is also subsequently amplified after it has been attached to the substrate.

Identifiers can be incorporated anywhere on a template. For example, a first identifier can be incorporated into a 5' end of the template and a second identifier can incorporated into a

3' end of the template. The first and second identifiers are designed such that each set of identifiers is correlated to a particular sample (e.g., material obtained from a particular patient or group of patients), allowing samples to be distinguished and/or validated. The first and second identifiers may be the same or different, so long as the combination is unique with respect to the sample or sub-sample that one seeks to distinguish and/or validate.

After identifiers have been incorporated into the template, the template is sequenced. Sequencing may be by any method known in the art. Sequencing-by-synthesis is a common technique used in next generation procedures and works well with the instant invention. However, other sequencing methods can be used, including sequence-by-ligation, sequencing-by-hybridization; gel-based techniques and others. In general, sequencing involves hybridizing a primer to a template to form a template/primer duplex, contacting the duplex with a polymerase in the presence of a detectably-labeled nucleotides under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner. Signal from the detectable label is then used to identify the incorporated base and the steps are sequentially repeated in order to determine the linear order of nucleotides in the template. Exemplary detectable labels include radiolabels, florescent labels, enzymatic labels, etc. In particular embodiments, the detectable label may be an optically detectable label, such as a fluorescent label. Exemplary fluorescent labels include cyanine, rhodamine, fluorescien, coumarin, BODIPY, alexa, or conjugated multi-dyes.

Numerous techniques are known for detecting sequence and for identifying barcodes and some are exemplified below. However, the exact means for detecting and compiling sequence data does not affect the function of the invention described herein.

If the analyte to be detected is not a nucleic acid, the identifiers can still be nucleic acid barcodes that are associated with the analyte binding moiety. Sequencing of the barcodes then validates detection of the analyte (i.e., the presence of two or more barcodes in association with the binder must be detected in order to validate the detection). In protein detection, the idenfiers can also be part of the primary structure of the protein to be detected. In this embodiment, the amino acid sequence of the protein is determined (e.g., by mass spectrometry or Edman degradation). The embedded amino acid sequence identifiers would have to be present in the amino acid sequence in order to validate the identity of the protein.

Brief Description of the Drawings

Figure 1 is a set of drawings showing how molecular overlap can lead to apparent high quality, but incorrect sequence data.

Figure 2 is a set of drawings depicting how methods of the invention allow one to detect, during analysis, whether the integrity of a patient's sample was maintained during a multiplex sequencing reaction.

Figure 3 is a graph showing that methods of the invention dramatically reduce false positives in a multiplex sequencing reaction.

Detailed Description

Methods of the invention relate to validating and maintaining the integrity of a nucleic acid template in a multiplex sequencing reaction. Methods of the invention involve obtaining a template nucleic acid, incorporating a pair of sequence identifiers into the template, and sequencing the template.

Reference is now made to Figure 1, which shows a set of drawings depicting one example of how molecular overlap can lead to apparent high quality, but incorrect sequence data. Panel 1 shows the pooling of nucleic acid libraries from two patient samples, A and B. Each template has a gene fragment from that patient (e.g., GA), and a molecular barcode specific to that patient (e.g., A). As shown in panel 1, nucleic acids from patient A's sample include a barcode A and a gene fragment GA, and nucleic acids from patient B's sample include nucleic a barcode B and a gene fragment GB.

Panel 2 shows that after pooling, the samples are then hybridized to primers at discrete locations on a flow cell. In this figure, the nucleic acid from patient A and the nucleic acid from patient B land within a cluster radius on the solid support. When the amplification process begins, primers attached to the solid support extend on each template. In one case (patient A's nucleic acid template), the primer extends across a full length of the template. In the other case (patient B's nucleic acid template), the primer partially extends, incorporating the sequence of barcode B and common priming sequence, but not the gene fragment sequence GB from patient B's nucleic acid template.

Panel 3 shows an example of improper molecular overlap. After extension, the duplexes are denatured and re-annealed for another amplification cycle. This example shows improper

molecular overlap via formation of a heteroduplex between patient A's nucleic acid template and patient B's nucleic acid template. In this example, strand *i* hybridizes to strand *ii*, forming a bridge. Barcode regions of each strand are not complementary, but the strands still hybridize because of the amount of common priming sequence on both sides of the barcode. Consequently, a polymerase will extend strand *ii*, copying patient A's gene fragment onto a strand containing patient B's barcode. A chimeric molecule has been formed.

Panel 4 shows the results of this molecular overlap. Strand *ii* contains the barcode for patient B but the gene fragment for patient A. During the sequencing process, patient A's sequence data will be improperly and incorrectly associated with patient B. If the chimeric molecule is formed early in the amplification process, it can dominate the cluster of amplification products, leading to a homogenous clonal chimeric cluster. During sequencing, this would give a high-quality signal, but the data will be assigned to the wrong sample because it has become associated with the wrong barcode.

Reference is now made to Figure 2, which shows a set of drawings depicting how methods of the invention allow one to detect, during analysis, whether the integrity of a patient's sample was maintained during a multiplex sequencing reaction. Panel 1 shows the pooling of nucleic acid libraries from two patient samples, A and B. Each template has a gene fragment from that patient (e.g., GA), and a pair of sequence identifiers (first and second) specific to that patient (e.g., A1 and A2).

The first and second identifiers are designed such that each set of identifiers is correlated to a particular patient, allowing patient samples to be distinguished. The first and second identifiers may be the same. Alternatively, the first and second identifiers may be different. In certain embodiments, the pair of identifiers are first and second barcode sequences that are attached to the template. The barcode sequences may be attached to the template such that a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template. The first and second barcode sequences may be the same, or they may be different.

In this figure, the sequence identifiers are pairs of barcode sequences. This figure also shows that the pair of barcode sequences are attached to the nucleic acid template at the 5' and the 3' ends of the template, such that the barcode sequences flank the gene fragment of each template. As shown in panel 1, the pool includes patient A's sample which is nucleic acid

templates having barcode sequences A1 and A2 that flank gene fragment GA, and also includes patient B's sample which is nucleic acid templates having barcode sequences B1 and B2 and gene fragment GB.

Panel 2 shows that after pooling, the samples are then hybridized to primers at discrete locations on a flow cell. In this figure, the nucleic acid from patient A and the nucleic acid from patient B land within a cluster radius on the solid support. When the amplification process begins, primers attached to the solid support extend on each template. In one case (patient A's nucleic acid template), the primer extends across a full length of the template. In the other case (patient B's nucleic acid template), the primer partially extends, incorporating the sequence of barcode B2 and common priming sequence, but not the gene fragment sequence GB from patient B's nucleic acid template or the sequence of barcode B1.

Panel 3 shows an example of improper molecular overlap. After extension, the duplexes are denatured and re-annealed for another amplification cycle. This example shows improper molecular overlap via formation of a heteroduplex between patient A's nucleic acid template and patient B's nucleic acid template. In this example, strand *i* hybridizes to strand *ii*, forming a bridge. Barcode regions A2 and B2 of each strand are not complementary, but the strands still hybridize because of the amount of common priming sequence on both sides of the barcode. Consequently, a polymerase will extend strand *ii*, copying patient A's gene fragment. However, in the case of two barcodes, only one of patient B's barcodes (B2) will be contained in the copied strand. The other barcode will be one of patient A's barcode sequences (A1). Unlike the example in Figure 1, the chimeric molecule formed in this example includes one barcode sequence from patient A (A1) and one barcode sequence from patient B (B2).

Panel 4 shows the results of this molecular overlap. While strand *ii* contains the gene fragment for patient A, it contains only one of patient B's barcodes (B2). The other barcode will be one of patient A's barcode sequences (A1). While each barcode sequence on its own is a valid barcode sequence, taken together, the two barcode sequences do not form a valid barcode sequence pair. During the sequencing process, this would yield a high-quality signal, but during data analysis the molecules would be recognized as invalid because the data contains an invalid barcode sequence pair, i.e., it is known that barcode sequence A1 should not be seen together with barcode sequence B2 and hence a crossover has occurred. Thus during analysis, it is

determined that the integrity of the patient's sample was not maintained and the data can then be excluded from final analysis.

Figure 3 is a graph showing that methods of the invention dramatically reduce false positives in a multiplex sequencing reaction. Two sample preparation procedures were performed prior to conducting a sequencing reaction as described herein. In the first sample preparation, only a single barcode sequence was attached to template molecules from different samples prior to pooling and then sequencing. In the second sample preparation, two barcode sequences were attached to template molecules from different samples prior to pooling and then sequencing.

Data in Figure 3 show that with use of only a single barcode sequence per template molecule, molecular overlap can lead to apparent high quality, but incorrect sequence data (diamond points). A single barcode sequence was attached to nucleic acids from sample A and a different single barcode sequence was attached to nucleic acids from sample B. The barcoded templates from each sample (A and B) were then pooled. After pooling, the samples were then hybridized to primers at discrete locations on a flow cell. Without being limited by any particular theory or mechanism of action, the data in Figure 3 suggest that the nucleic acid from sample A and the nucleic acid from sample B landed within a cluster radius on the solid support. When the amplification process began, primers attached to the solid support extended on each template. In some instances, the primer partially extended, incorporating the sequence of barcode B and common priming sequence, but not the gene fragment sequence of sample B.

After extension, the duplexes were denatured and re-annealed for another amplification cycle. In certain cases, a heteroduplex between sample A's nucleic acid template and sample B's nucleic acid template was formed. The barcode regions of each strand were not complementary, but the strands still hybridized because of the amount of common priming sequence on both sides of the barcode. Consequently, a polymerase extended the strand copying sample A's gene fragment onto a strand containing sample B's barcode. A chimeric molecule was formed. During the sequencing process, sample A's sequence data improperly and incorrectly associated with sample B. Since the chimeric molecule was formed early in the amplification process, it dominated the cluster of amplification products, leading to a homogenous clonal chimeric cluster. During sequencing, this gave a high-quality signal, but the data was assigned to the

wrong sample because it had become associated with the wrong barcode. See Figure 3, diamond points.

Figure 3 also provides data showing that methods of the invention dramatically reduce false positives in a multiplex sequencing reaction (square points). A pair of barcode sequences (A1 and A2 and B1 and B2) were attached to nucleic acids from two different samples, samples A and B. The pair of barcode sequences were attached to the nucleic acid templates at the 5' and the 3' ends of each template, such that the barcode sequences flanked the gene fragment of each template.

The barcoded templates from each sample (A and B) were then pooled. After pooling, the samples were then hybridized to primers at discrete locations on a flow cell. Without being limited by any particular theory or mechanism of action, the data in Figure 3 suggest that the nucleic acid from sample A and the nucleic acid from sample B landed within a cluster radius on the solid support. When the amplification process began, primers attached to the solid support extended on each template. In certain instances, the primer partially extended, incorporating the sequence of barcode B2 and common priming sequence, but not the gene fragment sequence from sample B's nucleic acid template or the sequence of barcode B1.

After extension, the duplexes were denatured and re-annealed for another amplification cycle. In this example, template from sample A and template from sample B hybridized, forming a bridge. Barcode regions A2 and B2 of each strand were not complementary, but the strands still hybridized because of the amount of common priming sequence on both sides of the barcode. Consequently, a polymerase extended the strand, copying sample A's gene fragment. However, in the case of two barcodes, only one of sample B's barcodes (B2) was contained in the copied strand. The other barcode was one of sample A's barcode sequences (A1). Unlike the single barcode amplification reaction, the chimeric molecule formed in this amplification reaction included one barcode sequence from sample A (A1) and one barcode sequence from sample B (B2).

While each barcode sequence on its own was a valid barcode sequence, taken together, the two barcode sequences did not form a valid barcode sequence pair. During the sequencing process, this yielded a high-quality signal, but during data analysis the molecules were recognized as invalid because the data contained an invalid barcode sequence pair, i.e., it was known that barcode sequence A1 should not be seen together with barcode sequence B2 and

hence a crossover has occurred. Thus during analysis, it was determined that the integrity of the patient's sample was not maintained and the data was excluded from final analysis (square points in Figure 3). Thus, data herein show that methods of the invention dramatically reduce crossover rate. High crossover rate makes it more likely that false positives and/or false negatives will occur.

The following sections discuss general considerations for barcode sequences, attaching barcode sequences to nucleic acid templates, and nucleic acid sequencing, for example, template considerations, polymerases useful in sequencing-by-synthesis, choice of surfaces, reaction conditions, signal detection and analysis.

Nucleic Acid Templates

Nucleic acid templates include deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA). Nucleic acid templates can be synthetic or derived from naturally occurring sources, or may include both synthetic and natural sequence; and may include PCR product. In one embodiment, nucleic acid template molecules are isolated from a biological sample containing a variety of other components, such as proteins, lipids and non-template nucleic acids. Nucleic acid template molecules can be obtained from any cellular material, obtained from an animal, plant, bacterium, fungus, or any other cellular organism. Biological samples for use in the present invention include viral particles or preparations. Nucleic acid template molecules can be obtained directly from an organism or from a biological sample obtained from an organism, e.g., from blood, urine, cerebrospinal fluid, seminal fluid, saliva, sputum, stool and tissue. Any tissue or body fluid specimen may be used as a source for nucleic acid for use in the invention. Nucleic acid template molecules can also be isolated from cultured cells, such as a primary cell culture or a cell line. The cells or tissues from which template nucleic acids are obtained can be infected with a virus or other intracellular pathogen. A sample can also be total RNA extracted from a biological specimen, a cDNA library, viral, or genomic DNA.

Nucleic acid obtained from biological samples typically is fragmented to produce suitable fragments for analysis. In one embodiment, nucleic acid from a biological sample is fragmented by sonication. Nucleic acid template molecules can be obtained as described in U.S. Patent Application Publication Number US2002/0190663 A1, published Oct. 9, 2003. Generally, nucleic acid can be extracted from a biological sample by a variety of techniques such as those

described by Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y., pp. 280-281 (1982). Generally, individual nucleic acid template molecules can be from about 1 base to about 20 kb. Nucleic acid molecules may be single-stranded, double-stranded, or double-stranded with single-stranded regions (for example, stem- and loop-structures).

A biological sample as described herein may be homogenized or fractionated in the presence of a detergent or surfactant. The concentration of the detergent in the buffer may be about 0.05% to about 10.0%. The concentration of the detergent can be up to an amount where the detergent remains soluble in the solution. In a preferred embodiment, the concentration of the detergent is between 0.1% to about 2%. The detergent, particularly a mild one that is nondenaturing, can act to solubilize the sample. Detergents may be ionic or nonionic. Examples of nonionic detergents include triton, such as the Triton® X series (Triton® X-100 t-Oct-C_6H_4-$(OCH_2$-$CH_2)_xOH$, x=9-10, Triton® X-100R, Triton® X-114 x=7-8), octyl glucoside, polyoxyethylene(9)dodecyl ether, digitonin, IGEPAL® CA630 octylphenyl polyethylene glycol, n-octyl-beta-D-glucopyranoside (betaOG), n-dodecyl-beta, Tween® 20 polyethylene glycol sorbitan monolaurate, Tween® 80 polyethylene glycol sorbitan monooleate, polidocanol, n-dodecyl beta-D-maltoside (DDM), NP-40 nonylphenyl polyethylene glycol, C12E8 (octaethylene glycol n-dodecyl monoether), hexaethyleneglycol mono-n-tetradecyl ether (C14EO6), octyl-beta-thioglucopyranoside (octyl thioglucoside, OTG), Emulgen, and polyoxyethylene 10 lauryl ether (C12E10). Examples of ionic detergents (anionic or cationic) include deoxycholate, sodium dodecyl sulfate (SDS), N-lauroylsarcosine, and cetyltrimethylammoniumbromide (CTAB). A zwitterionic reagent may also be used in the purification schemes of the present invention, such as Chaps, zwitterion 3-14, and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulf-onate. It is contemplated also that urea may be added with or without another detergent or surfactant.

Lysis or homogenization solutions may further contain other agents, such as reducing agents. Examples of such reducing agents include dithiothreitol (DTT), .beta.-mercaptoethanol, DTE, GSH, cysteine, cysteamine, tricarboxyethyl phosphine (TCEP), or salts of sulfurous acid.

Barcode Sequences

In certain embodiments, the sequence identifiers are barcode sequences that are attached to or incorporated into a nucleic acid template. The barcode sequences may be attached to the

template such that a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template. The first and second barcode sequences may be the same, or they may be different. Barcode sequence may be incorporated into a contiguous region of a template that includes the target to be sequenced.

Exemplary methods for designing sets of barcode sequences and other methods for attaching barcode sequences are shown in U.S. paten numbers 6,138,077; 6,352,828; 5,636,400; 6,172,214; 6235,475; 7,393,665; 7,544,473; 5,846,719; 5,695,934; 5,604,097; 6,150,516; RE39,793; 7,537,897; 6172,218; and 5,863,722, the content of each of which is incorporated by reference herein in its entirety.

The barcode sequence generally includes certain features that make the sequence useful in sequencing reactions. For example the barcode sequences can be designed to have minimal or no homopolymer regions, i.e., 2 or more of the same base in a row such as AA or CCC, within the barcode sequence. The barcode sequences can also be designed so that they do not overlap the target region to be sequence or contain a sequence that is identical to the target.

The first and second barcode sequences are designed such that each pair of sequences is correlated to a particular sample, allowing samples to be distinguished and validated. Methods of designing sets of barcode sequences is shown for example in Brenner et al. (U.S. patent number 6,235,475), the contents of which are incorporated by reference herein in their entirety. In certain embodiments, the barcode sequences range from about 2 nucleotides to about 50; and preferably from about 4 to about 20 nucleotides. Since the barcode sequence is sequenced along with the template nucleic acid or may be sequenced in a separate read, the oligonucleotide length should be of minimal length so as to permit the longest read from the template nucleic acid attached. Generally, the barcode sequences are spaced from the template nucleic acid molecule by at least one base.

Methods of the invention involve attaching the barcode sequences to the template nucleic acids. Template nucleic acids are able to be fragmented or sheared to desired length, e.g. generally from 100 to 500 bases or longer, using a variety of mechanical, chemical and/or enzymatic methods. DNA may be randomly sheared via sonication, exposed to a DNase or one or more restriction enzymes, a transposase, or nicking enzyme. RNA may be fragmented by brief exposure to an RNase, heat plus magnesium, or by shearing. The RNA may be converted to cDNA before or after fragmentation.

Barcode sequence is integrated with template using methods known in the art. Barcode sequence is integrated with template using, for example, a ligase, a polymerase, Topo cloning (e.g., Invitrogen's topoisomerase vector cloning system using a topoisomerase enzyme), or chemical ligation or conjugation. The ligase may be any enzyme capable of ligating an oligonucleotide (RNA or DNA) to the template nucleic acid molecule. Suitable ligases include T4 DNA ligase and T4 RNA ligase (such ligases are available commercially, from New England Biolabs). Methods for using ligases are well known in the art. The polymerase may be any enzyme capable of adding nucleotides to the 3' and the 5' terminus of template nucleic acid molecules. Barcode sequence can be incorporated via a PCR reaction as part of the PCR primer.

The ligation may be blunt ended or via use of over hanging ends. In certain embodiments, following fragmentation, the ends of the fragments may be repaired, trimmed (e.g. using an exonuclease), or filled (e.g., using a polymerase and dNTPs), to form blunt ends. Upon generating blunt ends, the ends may be treated with a polymerase and dATP to form a template independent addition to the 3'-end and the 5-end of the fragments, thus producing a single A overhanging. This single A is used to guide ligation of fragments with a single T overhanging from the 5'-end in a method referred to as T-A cloning.

Alternatively, because the possible combination of overhangs left by the restriction enzymes are known after a restriction digestion, the ends may be left as is, i.e., ragged ends. In certain embodiments double stranded oligonucleotides with complementary over hanging ends are used.

Surface Attachment

Methods of the invention may involve attaching or immobilizing barcoded nucleic acid templates to solid supports. Such methods are described for example in Sabot et al. (U.S. patent application number 2009/0226975), Adessi et al. (U.S. patent number 7,115,400), and Kawashima et al. (U.S. patent application number 2005/0100900), the content of each of which is incorporated by reference herein in its entirety.

The term immobilized as used herein is intended to encompass direct or indirect attachment to a solid support via covalent or non-covalent bond(s). In certain embodiments of the invention, covalent attachment may be used, but generally all that is required is that template remain immobilized on the support. Typically oligonucleotides are immobilized such that a 3'

end is available for enzymatic extension and at least a portion of the sequence is capable of hybridizing to a complementary sequence. Immobilization can occur via hybridization to a surface attached oligonucleotide. Alternatively, immobilization can occur by means other than base-pairing hybridization, such as the covalent attachment set forth above.

Substrates or supports for use in the invention include, but are not limited to, latex beads, dextran beads, polystyrene surfaces, polypropylene surfaces, polyacrylamide gel, gold surfaces, glass surfaces and silicon wafers. In certain embodiments, the solid support may include an inert substrate or matrix that has been functionalized, for example by the application of a layer or coating of an intermediate material including reactive groups that permit covalent attachment to molecules such as polynucleotides.

Amplification

In certain embodiments, methods of the invention involve amplifying barcoded nucleic acid templates prior to sequencing the templates. Such methods are described for example in Sabot et al. (U.S. patent application number 2009/0226975), Adessi et al. (U.S. patent number 7,115,400), and Kawashima et al. (U.S. patent application number 2005/0100900), the content of each of which is incorporated by reference herein in its entirety.

Primer oligonucleotides or amplification sequences are polynucleotide sequences that are capable of annealing specifically to a single stranded polynucleotide sequence to be amplified under conditions encountered in a primer annealing step of an amplification reaction. Generally, the terms nucleic acid, polynucleotide and oligonucleotide are used interchangeably herein. The different terms are not intended to denote any particular difference in size, sequence, or other property unless specifically indicated otherwise. For clarity of description the terms may be used to distinguish one species of molecule from another when describing a particular method or composition that includes several molecular species.

Primers may additionally include non-nucleotide chemical modifications, for example to facilitate covalent attachment of the primer to a solid support. Certain chemical modifications may themselves improve the function of the molecule as a primer or may provide some other useful functionality, such as providing a cleavage site that enables the primer (or an extended polynucleotide strand derived therefrom) to be cleaved from a solid support. Useful chemical modifications can also provide reversible modifications that prevent hybridization or extension

of the primer until the modification is removed or reversed. Similarly, other molecules attached to a surface in accordance with the invention can include cleavable linker moieties and or reversible modifications that alter a particular chemical activity of function of the molecule.

A plurality of oligonucleotides used in the methods set forth herein can include species that function as capture oligonucleotides. The capture oligonucleotides may include a template specific portion, namely a sequence of nucleotides capable of annealing to a primer binding sequence in a single stranded polynucleotide molecule of interest such as one that is to be amplified. The primer binding sequences will generally be of known sequence and will therefore be complementary to a region of known sequence of the single stranded polynucleotide molecule. The capture oligonucleotides may include a capture sequence and an amplification sequence. For example, a capture oligonucleotide may be of greater length than amplification primers that are attached to the same substrate, in which case the 5' end of the capture sequences may comprise a region with the same sequence as one of the amplification primers. A portion of a template, such as the 3' end of the template, may be complementary to the 3' of the capture sequences. The 5' end of the template may contain a region that comprises a sequence identical to one of the amplification primers such that upon copying the template, the copy can hybridize to the immobilized amplification primer. Thus, an oligonucleotide species that is useful in the methods set forth herein can have a capture sequence, an amplification sequence or both. Conversely, an oligonucleotide species can lack a capture sequence, an amplification sequence or both. In this way the hybridization specificity of an oligonucleotide species can be tailored for a particular application of the methods.

The length of primer binding sequences need not be the same as those of known sequences of polynucleotide template molecules and may be shorter, being particularly 16-50 nucleotides, more particularly 16-40 nucleotides and yet more particularly 20-30 nucleotides in length. The desired length of the primer oligonucleotides will depend upon a number of factors. However, the primers are typically long (complex) enough so that the likelihood of annealing to sequences other than the primer binding sequence is very low. Accordingly, known sequences that flank a template sequence can include a primer binding portion and other portions such as a capture sequence, barcode sequence or combination thereof.

In certain embodiments of the invention, amplification primers for solid phase amplification are immobilized by covalent attachment to the solid support at or near the 5' end of

the primer, such that a portion of the primer is free to anneal to its cognate template and the 3' hydroxyl group is free to function in primer extension.

The chosen attachment chemistry will typically depend on the nature of the solid support and any functionalization or derivatization applied to it. In the case of nucleic acid embodiments, the primer itself may include a moiety which may be a non-nucleotide chemical modification to facilitate attachment. For example, the primer may include a sulfur containing nucleophile such as a phosphorothioate or thiophosphate at the 5' end. In the case of solid supported polyacrylamide hydrogels, this nucleophile may bind to a bromoacetamide group present in the hydrogel. In one embodiment, the means of attaching primers to the solid support is via St phosphorothioate attachment to a hydrogel comprised of polymerized acrylamide and N-(5-bromoacetamidylpentyl) acrylamide (BRAPA).

A uniform, homogeneously distributed lawn of immobilized oligonucleotides may be formed by coupling (grafting) a solution of oligonucleotide species onto the solid support. The solution can contain a homogenous population of oligonucleotides but will typically contain a mixture of different oligonucleotide species. The mixture can include, for example, at least two, three or more different species of oligonucleotide. Each surface that is exposed to the solution therefore reacts with the solution to create a uniform density of immobilized sequences over the whole of the exposed solid support. As such, a portion of the surface having a mixture of different immobilized sequences can be surrounded by an area of the surface having a mixture of the same immobilized sequences. A suitable density of amplification oligonucleotides is at least 1 fmol/mm^2 (6 x 10^{10} per cm^2), or more optimally at least 10 fmol/mm^2 (6 x 10^{11} per cm^2). The density of the capture oligonucleotides can be controlled to give an optimum cluster density of 10^6 - 10^9 clusters per cm^2 and optimum cluster brightness. The ratio of capture oligonucleotide species to the amplification oligonucleotide species can be any desired value including, but not limited to at least 1:100, 1:1000 or 1:100000 depending on the desired cluster density and brightness. Similar densities or ratios of other molecular species can be used in embodiments where molecules other than nucleic acids are attached to a surface.

In a particular embodiment, for each cluster of template molecules, a complementary copy of a single stranded polynucleotide template molecule is attached to the solid support by hybridization. Methods of hybridization for formation of stable duplexes between complementary sequences by way of Watson-Crick base-pairing are known in the art. The

immobilized capture oligonucleotides can include a region of sequence that is complementary to a region or template specific portion of the single stranded template polynucleotide molecule. An extension reaction may then be carried out in which the capture sequence is extended by sequential addition of nucleotides to generate a complementary copy of the single stranded polynucleotide sequence attached to the solid support via the capture oligonucleotide. The single stranded polynucleotide sequence not immobilized to the support may be separated from the complementary sequence under denaturing conditions and removed, for example by washing.

The terms separate and separating, when used in reference to strands of a nucleic acid, refer to the physical dissociation of the DNA bases that interact within for example, a Watson-Crick DNA-duplex of the single stranded polynucleotide sequence and its complement. The terms also refer to the physical separation of these strands. Thus, the term can refer to the process of creating a situation wherein annealing of another primer oligonucleotide or polynucleotide sequence to one of the strands of a duplex becomes possible. After the first extension reaction, the duplex is immobilized through a single 5' attachment, and hence strand separation can result in loss of one of the strands from the surface. In cases where both strands of the duplex are immobilized, separation of the strands means that the duplex is converted into two immobilized single strands.

In one aspect of the invention, one or more of the amplification primers can be modified to prevent hybridization of a region or template specific portion of the single stranded polynucleotide molecule. Alternatively or additionally, one or more of the amplification primers may be modified to prevent extension of the primer during one or more extension reactions, thus preventing copying of the hybridized templates. These modifications can be temporary or permanent.

Generally, the capture sequences will include a region of the same sequence as the plurality of amplification oligonucleotides. Once the 3' end of the extended immobilized template copy has hybridized to one of the amplification primers and been extended, the resulting duplex will be immobilized at both ends and all of the bases in the capture oligonucleotide sequence will have been copied. Thus the capture oligonucleotide may include both the amplification primer sequence, plus a further sequence that is complementary to the end of the template. Typically the sequence complementary to the end of the template will not be present in any of the amplification primers. Alternatively, the amplification primers can contain

the sequences complementary to the ends of the single stranded templates, but the amplification primers can be reversibly blocked to prevent hybridization and/or extension during one or more extension step, such as a first extension step in a particular amplification process.

According to one aspect of the invention, one or more of the amplification primers may include a modification that acts as a reversible block to either template hybridization or extension or both. By way of non-limiting example, such modifications can be presence of an additional sequence of nucleotides that is complementary to the amplification primer. This additional sequence can be present in a portion of the amplification primer and thus acts as an intramolecular hairpin duplex, or a 3' blocking group that prevents extension of the primer. Alternatively, the additional sequence can be found on a separate oligonucleotide that hybridizes to the amplification primer. A particular feature of such a modification is that it can be removed, altered or reversed such that the functionality of the modified primer oligonucleotide is restored and the primer is able to undergo hybridization and extension during later steps of the methods. Among other examples, the blocking group may be a small chemical species such as a 3' phosphate moiety that can be removed enzymatically, may be an a basic nucleotide such that the 3' end of the primer is not capable of hybridization (and thereby extension), or may be a sequence of nucleotides that can be selectively excised from the immobilized strands, for example, using restriction endonucleases that selectively cleave particular sequences or deglycosylases that selectively cleave oligonucleotides having exogenous bases such as uracil deoxyribonucleotides or 8-oxoguanine.

In one embodiment a plurality of three types of oligonucleotides (for example comprising capture sequences, forward and reverse primers) are immobilized to a solid support. Alternatively the three oligonucleotides may be forward amplification, blocked forward amplification and reverse amplification, where the unblocked forward primer acts as the capture sequence.

The single stranded polynucleotide molecules may have originated in single-stranded form, as DNA or RNA or may have originated in double-stranded DNA (dsDNA) form (e.g. genomic DNA fragments, PCR and amplification products and the like). Thus a single stranded polynucleotide may be the sense or antisense strand of a polynucleotide duplex. Methods of preparation of single stranded polynucleotide molecules suitable for use in the method of the invention using standard techniques are well known in the art. The precise sequence of the

primary polynucleotide molecules may be known or unknown during different steps of the methods set forth herein. It will be understood that a double stranded polynucleotide molecule can be hybridized to an immobilized capture oligonucleotide as exemplified herein for single stranded polynucleotide molecules, so long as a single stranded region of the double stranded polynucleotide is available and at least partially complementary to the capture oligonucleotide sequence.

An exemplary method for the isolation of one strand of a double stranded molecular construct is described herein. A sample of unknown sequence may be fragmented and have barcode sequences attached at both ends of the fragment. Adapters are then attached to the ends of each fragment. One strand of the adapters may contain a moiety for surface immobilization, for example a biotin that can be captured onto a streptavidin surface. The adapters may be mismatch adapters, for example as described in co-pending application US 2007/0128624, the contents of which are incorporated herein by reference in their entirety. Amplification of the mismatch or forked adapters using a pair of amplification primers, one of which carries a biotin modification means that one strand of each duplex carries a biotin modification. Immobilization of the strands onto a streptavidin surface means that the non-biotinylated strand can be eluted simply by denaturation/strand separation. The eluted constructs will be in single stranded form and upon exposure to hybridization conditions can be used to hybridize against the immobilized capture sequences which can be extended.

In a particular embodiment, the single stranded polynucleotide molecules are DNA molecules. More particularly, the single stranded polynucleotide molecules represent genomic DNA molecules, or amplicons thereof, which include both intron and exon sequence (coding sequence), as well as non-coding regulatory sequences such as promoter and enhancer sequences. Still yet more particularly, the single stranded polynucleotide molecules are human genomic DNA molecules, or amplicons thereof.

In a particular embodiment, a single stranded target polynucleotide molecule has at least two regions of known sequence. Yet more particularly, the 5' and 3' termini of the single stranded polynucleotide molecule will have the regions of known sequence such that the single stranded polynucleotide molecule will be of the structure:

5'[known sequence I]-[barcode sequence]-[known sequence II]-[target polynucleotide sequence]-[known sequence III]-[barcode sequence]-[known sequence IV]-3'

Typically "known sequence I", "known sequence II", "known sequence III", and "known sequence IV" will consist of more than 20, or more than 40, or more than 50, or more than 100, or more than 300 consecutive nucleotides. The precise length of the four sequences may or may not be identical. The primer binding sequences generally will be of known sequence and will therefore particularly be complementary to a sequence within “"known sequence I", "known sequence II", "known sequence III", and "known sequence IV" of the single stranded polynucleotide molecule. The length of the primer binding sequences need not be the same as those of known sequence I-IV, and may be shorter, being particularly 16-50 nucleotides, more particularly 16-40 nucleotides and yet more particularly 20-30 nucleotides in length. Known sequences I-IV can be the same or different for each other.

Methods of hybridization for formation of stable duplexes between complementary sequences by way of Watson-Crick base pairing are known in the art. A region or part of the single stranded polynucleotide template molecules can be complementary to at least a part of the immobilized capture sequence oligonucleotides. Since the amplification oligonucleotides are either modified to prevent hybridization and/or extension, or are non-complementary to the known ends of the template strands, only the capture sequences will be capable of hybridization and extension. An extension reaction may then be carried out wherein the capture sequence primer is extended by sequential addition of nucleotides to generate a complementary copy of the single stranded template polynucleotide attached to the solid support via the capture sequence oligonucleotide. The single stranded template polynucleotide sequence not immobilized to the support may be separated from the complementary sequence under denaturing conditions and removed, for example by washing. The distance between the individual capture sequence oligonucleotides on the surface therefore controls the density of the single stranded template polynucleotides and hence the density of clusters formed later on the surface is also controlled.

In certain embodiments in which the modified forward primer oligonucleotides are blocked and are unable to be extended, generally all of the amplification primer oligonucleotides will hybridize to the single stranded template polynucleotides. When the extension reaction is carried out only the unmodified forward capture primer oligonucleotides are extended by

sequential addition of nucleotides to generate a complementary copy of the single stranded template polynucleotide attached to the solid support via the unmodified forward primer oligonucleotide. The single stranded template polynucleotide sequences not hybridized to the support may be separated from the un-extended blocked forward primer oligonucleotides under denaturing conditions and removed, for example by washing with a chemical denaturant such as formamide. The distance between the individual unmodified forward primer oligonucleotides on the surface therefore controls the density of the single stranded template polynucleotides and hence the density of clusters formed later on the surface is also controlled.

Following the attachment of the complementary single stranded template polynucleotides, the modified/blocked primers can be treated to reverse, remove or alter the modification such that they become functionally equivalent to the unmodified forward primer oligonucleotides. For example, the double stranded structure may be removed either by denaturation, for example by heating or treatment with an alkaline solution when it is formed by a separate hybridized polynucleotide. Alternatively, where the hybridized polynucleotide is covalently linked, enzymatic digestion could be used to sequence-selectively cleave the strand, followed by denaturation. Such methods for removing the double stranded structure are known in the art and would be apparent to the skilled person (Sambrook and Russell, Molecular Cloning, A Laboratory Manual, third edition, Cold Spring Harbor Laboratory Press (2001)).

In one embodiment of the invention, the single stranded template polynucleotide molecule can be attached to the solid support by ligation to double stranded primers immobilized to the solid support using ligation methods known in the art (Sambrook and Russell, supra). Such methods utilize ligase enzymes such as DNA ligase to effect or catalyze the joining of the ends of the two polynucleotide strands, in this case, the single stranded template polynucleotide molecule and the primer oligonucleotide ligate such that covalent linkages are formed. In this context "joining" means covalent linkage of two polynucleotide strands that were not previously covalently linked. Thus, an aim of the invention can also be achieved by modifying the 3' end of a subset of primer oligonucleotides such that they are unable to ligate to the single stranded template polynucleotides. By way of non-limiting example, the addition of 2'3'dideoxy AMP (dideoxyAMP) by the enzyme terminal deoxynucleotidyl transferase (TdT) effectively prevents T4 DNA ligase from ligating treated molecules together.

An alternative method would be to have the capture sequences as duplex strands and the

amplification sequences as single strands. Upon ligation of the single strands to the capture duplexes (which would be the only immobilized species carrying a free 5' phosphate) the 3' end of the immobilized strand can be extended as described above. Upon denaturation of the hybridized template sequence, amplification of the immobilized strand can proceed as described. Other such methods for attaching single strands will be apparent to others skilled in the art.

In a next step according to particular embodiments of the present invention, suitable conditions are applied to the immobilized single stranded polynucleotide molecule and the plurality of amplification primer oligonucleotides such that the single stranded polynucleotide molecule hybridizes to an amplification primer oligonucleotide to form a complex in the form of a bridge structure. Suitable conditions such as neutralizing and/or hybridizing buffers are well known in the art (See Sambrook et al., supra; Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1998)). The neutralising and/or hybridising buffer may then be removed.

Next by applying suitable conditions for extension an extension reaction is performed. The primer oligonucleotide of the complex is extended by sequential addition of nucleotides to generate an extension product complimentary to the single stranded polynucleotide molecule. The resulting duplex is immobilized at both 5' ends such that each strand is immobilized.

Suitable conditions such as extension buffers/solutions comprising an enzyme with polymerase activity are well known in the art (See Sambrook et al., supra; Ausubel et al. supra). In a particular embodiment dNTP's may be included in the extension buffer. In a further embodiment dNTP's could be added prior to the extension buffer. This bridge amplification technique can be carried out as described, for example, in Adessi et al. (U.S. patent number 7,115,400), and Kawashima et al. (U.S. patent application number 2005/0100900), the contents of which are incorporated herein by reference.

Examples of enzymes with polymerase activity which can be used in the present invention are DNA polymerase (Klenow fragment, T4 DNA polymerase), heat-stable DNA polymerases from a variety of thermostable bacteria (such as Taq, VENT, Pfu, or Tfl DNA polymerases) as well as their genetically modified derivatives (TaqGold, VENTexo, or Pfu exo). A combination of RNA polymerase and reverse transcriptase can also be used to generate the extension products. Particularly the enzyme has strand displacement activity, more particularly the enzyme will be active at a pH of about 7 to about 9, particularly pH 7.9 to pH 8+, yet more

particularly the enzymes are Est or Klenow.

The nucleoside triphosphate molecules used are typically deoxyribonucleotide triphosphates, for example dATP, dTTP, dCTP, dGTP, or are ribonucleoside triphosphates for example ATP, UTP, CTP, GTP, The nucleoside triphosphate molecules may be naturally or non-naturally occurring.

After the hybridization and extension steps, the support and attached nucleic acids can be subjected to denaturation conditions. A flow cell can be used such that, the extension buffer is generally removed by the influx of the denaturing buffer. Suitable denaturing buffers are well known in the art (See Sambrook et al., supra; Ausubel et al. supra). By way of example it is known that alterations in pH and low ionic strength solutions can denature nucleic acids at substantially isothermal temperatures. Formamide and urea form new hydrogen bonds with the bases of nucleic acids disrupting hydrogen bonds that lead to Watson-Crick base pairing. In a particular embodiment the concentration of formamide is 50% or more. These result in single stranded nucleic acid molecules. If desired, the strands may be separated by treatment with a solution of very low salt (for example less than 0.01 M cationic conditions) and high pH (>12) or by using a chaotropic salt (e.g. guanidinium hydrochloride). In a particular embodiment a strong base is used. A strong base is a basic chemical compound that is able to deprotonate very weak acids in an acid base reaction. The strength of a base is indicated by its pK.sub.b value, compounds with a pK_b value of less than about 1 are called strong bases and are well known to one skilled in the art. In a particular embodiment the strong base is Sodium Hydroxide (NaOH) solution used at a concentration of from 0.05 M to 0.25 M, particularly 0.1 M.

Following the hybridization, extension and denaturation steps exemplified above, two immobilized nucleic acids will be present, the first being the first template single stranded polynucleotide molecule (that was initially immobilized) and the second being a nucleic acid complementary thereto, extending from one of the immobilized primer oligonucleotides. Both the original immobilized single stranded polynucleotide molecule and the immobilized extended primer oligonucleotide formed are then able to initiate further rounds of amplification by subjecting the support to further cycles of hybridization, extension and denaturation.

It may be advantageous to perform optional washing steps in between each step of the amplification method. For example an extension buffer without polymerase enzyme with or without dNTP's could be applied to the solid support before being removed and replaced with the

full extension buffer.

Such further rounds of amplification can be used to produce a nucleic acid colony or cluster comprising multiple immobilized copies of the single stranded polynucleotide sequence and its complementary sequence.

The initial immobilization of the single stranded polynucleotide molecule means that the single stranded polynucleotide molecule can hybridize with primer oligonucleotides located at a distance within the total length of the single stranded polynucleotide molecule. Other surface bound primers that are out of reach will not hybridize to the polynucleotide. Thus the boundary of the nucleic acid colony or cluster formed is limited to a relatively local area surrounding the location in which the initial single stranded polynucleotide molecule was immobilized.

Once more copies of the single stranded polynucleotide molecule and its complement have been synthesized by carrying out further rounds of amplification, i.e. further rounds of hybridization, extension and denaturation, then the boundary of the nucleic acid colony or cluster being generated will be able to be extended further, although the boundary of the colony formed is still limited to a relatively local area around the location in which the initial single stranded polynucleotide molecule was immobilized. For example the size of each amplified cluster may be 0.5-5 microns.

It can thus be seen that the method of the present invention allows the generation of a plurality of nucleic acid colonies from multiple single immobilized single stranded polynucleotide molecules and that the density of these colonies can be controlled by altering the proportions of modified capture/amplification oligonucleotides used to graft the surface of the solid support.

In one embodiment, the hybridization, extension and denaturation steps are all carried out at the same, substantially isothermal temperature. For example the temperature is from 37°C to about 75°C, particularly from 50°C to 70°C, yet more particularly from 60°C to 65°C. In a particular embodiment the substantially isothermal temperature may be the optimal temperature for the desired polymerase.

In a particular aspect, the method according to the first aspect of the invention is used to prepare clustered arrays of nucleic acid colonies, analogous to those described in U.S. Pat. No. 7,115,400, US 2005/0100900 A1, WO 00/18957 and WO 98/44151 (the contents of which are herein incorporated by reference), by solid-phase amplification.

In yet another aspect more than one capture sequences and more than two amplification sequences, for example, at least three or four or more, different amplification primer sequences may be grafted to the solid support. In this manner more than one library, with common sequences which differ between the libraries, could be utilized to prepare clusters, such as, for example libraries prepared from two different patients. Whilst the cluster may overlap in space, they would be able to be sequenced one after the other due to the differences between the ends of the templates. For example, two different samples can be captured using two different capture sequences. These can be amplified from the same two amplification primers. The samples can be differentiated due to the two different capture sequences, which can be used as the sites for hybridization of two different sequencing primers. The use of different capture sequences thereby gives rise to a method of sample indexing using different sequencing primers.

Clustered arrays formed by the methods of the invention are suitable for use in applications usually carried out on ordered arrays such as micro-arrays. Such applications by way of non-limiting example include hybridization analysis, gene expression analysis, protein binding analysis, sequencing, genotyping, nucleic acid methylation analysis and the like. The clustered array may be sequenced before being used for downstream applications such as, for example, hybridization with fluorescent RNA or binding studies using fluorescent labelled proteins.

Sequencing Methods

The invention also encompasses methods of sequencing amplified nucleic acids generated by solid-phase amplification. Thus, the invention provides a method of nucleic acid sequencing comprising amplifying a pool of nucleic acid templates using solid-phase amplification as described above and carrying out a nucleic acid sequencing reaction to determine the sequence of the whole or a part of at least one amplified nucleic acid strand produced in the solid-phase amplification reaction.

Sequencing can be carried out using any suitable sequencing technique. A particularly useful method is one wherein nucleotides are added successively to a free 3’ hydroxyl group, resulting in synthesis of a polynucleotide chain in the 5' to 3' direction. The nature of the nucleotide added may be determined after each nucleotide addition or at the end of the sequencing process. Sequencing techniques using sequencing by ligation, wherein not every

contiguous base is sequenced, and techniques such as massively parallel signature sequencing (MPSS) where bases are removed from, rather than added to the strands on the surface are also within the scope of the invention.

The initiation point for the sequencing reaction may be provided by annealing of a sequencing primer to a product of the solid-phase amplification reaction. In this connection, one or both of the adaptors added during formation of the template library may include a nucleotide sequence which permits annealing of a sequencing primer to amplified products derived by whole genome or solid-phase amplification of the template library.

The products of solid-phase amplification reactions wherein both forward and reverse amplification primers are covalently immobilized on the solid surface are so-called bridged structures formed by annealing of pairs of immobilized polynucleotide strands and immobilized complementary strands, both strands being attached to the solid support at the 5' end. Arrays comprised of such bridged structures provide inefficient templates for typical nucleic acid sequencing techniques, since hybridization of a conventional sequencing primer to one of the immobilized strands is not favored compared to annealing of this strand to its immobilized complementary strand under standard conditions for hybridization.

In order to provide more suitable templates for nucleic acid sequencing, it may be advantageous to remove or displace substantially all or at least a portion of one of the immobilized strands in the bridged structure in order to generate a template which is at least partially single-stranded. The portion of the template which is single-stranded will thus be available for hybridization to a sequencing primer. The process of removing all or a portion of one immobilized strand in a `bridged` double-stranded nucleic acid structure may be referred to herein as linearization, and is described in further detail in WO07010251, the contents of which are incorporated herein by reference in their entirety.

Bridged template structures may be linearized by cleavage of one or both strands with a restriction endonuclease or by cleavage of one strand with a nicking endonuclease. Other methods of cleavage can be used as an alternative to restriction enzymes or nicking enzymes, including inter alia chemical cleavage (e.g. cleavage of a diol linkage with periodate), cleavage of abasic sites by cleavage with endonuclease (for example `USER`, as supplied by NEB, part number M5505S), or by exposure to heat or alkali, cleavage of ribonucleotides incorporated into amplification products otherwise comprised of deoxyribonucleotides, photochemical cleavage or

cleavage of a peptide linker.

Following the cleavage step, regardless of the method used for cleavage, the product of the cleavage reaction may be subjected to denaturing conditions in order to remove the portion(s) of the cleaved strand(s) that are not attached to the solid support. Suitable denaturing conditions, for example sodium hydroxide solution, formamide solution or heat, will be apparent to the skilled reader with reference to standard molecular biology protocols (Sambrook et al., supra; Ausubel et al. supra). Denaturation results in the production of a sequencing template which is partially or substantially single-stranded. A sequencing reaction may then be initiated by hybridization of a sequencing primer to the single-stranded portion of the template.

Thus, the invention encompasses methods wherein the nucleic acid sequencing reaction comprises hybridizing a sequencing primer to a single-stranded region of a linearized amplification product, sequentially incorporating one or more nucleotides into a polynucleotide strand complementary to the region of amplified template strand to be sequenced, identifying the base present in one or more of the incorporated nucleotide(s) and thereby determining the sequence of a region of the template strand.

One sequencing method which can be used in accordance with the invention relies on the use of modified nucleotides having removable 3' blocks, for example as described in WO04018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, the contents of which are incorporated herein by reference in their entirety. Once the modified nucleotide has been incorporated into the growing polynucleotide chain complementary to the region of the template being sequenced there is no free 3'-OH group available to direct further sequence extension and therefore the polymerase can not add further nucleotides. Once the nature of the base incorporated into the growing chain has been determined, the 3' block may be removed to allow addition of the next successive nucleotide. By ordering the products derived using these modified nucleotides, it is possible to deduce the DNA sequence of the DNA template. Such reactions can be done in a single experiment if each of the modified nucleotides has a different label attached thereto, known to correspond to the particular base, to facilitate discrimination between the bases added during each incorporation step. Alternatively, a separate reaction may be carried out containing each of the modified nucleotides separately.

The modified nucleotides may carry a label to facilitate their detection. A fluorescent label, for example, may be used for detection of modified nucleotides. Each nucleotide type may

thus carry a different fluorescent label, for example, as described in WO07135368, the contents of which are incorporated herein by reference in their entirety. The detectable label need not, however, be a fluorescent label. Any label can be used which allows the detection of an incorporated nucleotide.

One method for detecting fluorescently labeled nucleotides comprises using laser light of a wavelength specific for the labelled nucleotides, or the use of other suitable sources of illumination. The fluorescence from the label on the nucleotide may be detected by a CCD camera or other suitable detection means. Suitable instrumentation for recording images of clustered arrays is described in WO07123744, the contents of which are incorporated herein by reference in their entirety.

The invention is not intended to be limited to use of the sequencing method outlined above, as essentially any sequencing methodology which relies on successive incorporation of nucleotides into a polynucleotide chain can be used. Suitable alternative techniques include, for example, the Genome Sequencers from Roche/454 Life Sciences (Margulies et al. (2005) Nature, 437:376-380; U.S. Pat. Nos. 6,274,320; 6,258,568; 6,210,891), and the SOLiD system from Applied Biosystems (solid.appliedbiosystems.com), and the sequencer from Ion Torrent (www.iontorrent.com).

Incorporation by Reference

References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made throughout this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein.

What is claimed is:

1. A method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction, the method comprising:

obtaining a template nucleic acid;

incorporating at least two sequence identifiers into the template; and

sequencing the template.

2. The method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

3. The method according to claim 2, wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

4. The method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

5. The method according to claim 2, wherein the first and second identifiers are the same.

6. The method according to claim 2, wherein the first and second identifiers are different.

7. The method according to claim 1, wherein prior to sequencing, the template is attached to a substrate.

8. The method according to claim 2, wherein the template is directly attached to the substrate.

9. The method according to claim 2, wherein the template is indirectly attached to the substrate.

10. The method according to claim 7, further comprising, amplifying the template.

11. The method according to claim 1, wherein sequencing is sequencing by synthesis.

12. The method according to claim 11, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

13. The method according to claim 1, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

14. The method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

15. The method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

16. The method according to claim 1, wherein the pair of identifiers are first and second barcode sequences that are attached to the template.

17. The method according to claim 16, wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template.

18. The method according to claim 17, wherein the first and second barcode sequences are the same.

19. The method according to claim 17, wherein the first and second barcode sequences are different.

20. A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a pair of barcode sequences to the template; and

sequencing the template.

21. The method according to claim 20, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

22. The method according to claim 21, wherein after attaching of the pair of barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

23. The method according to claim 20, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

24. The method according to claim 20, wherein prior to sequencing, the template is attached to a substrate.

25. The method according to claim 24, wherein the template is directly attached to the substrate.

26. The method according to claim 24, wherein the template is indirectly attached to the substrate.

27. The method according to claim 24, further comprising, amplifying the template.

28. The method according to claim 20, wherein sequencing is sequencing by synthesis.

29. The method according to claim 28, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

30. The method according to claim 20, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

31. The method according to claim 29, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

32. The method according to claim 31, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

33. The method according to claim 23, wherein the first and second barcode sequences are the same.

34. The method according to claim 23, wherein the first and second barcode sequences are different.

35. A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template;

amplifying the template; and

sequencing the template.

36. The method according to claim 35, wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

37. The method according to claim 35, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

38. The method according to claim 35, wherein prior to amplifying, the template is immobilized to a solid support.

39. The method according to claim 35, wherein the first and second barcode sequences are the same.

40. The method according to claim 35, wherein the first and second barcode sequences are different.

41. The method according to claim 35, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

42. A method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte.

43. The method of claim 42, wherein said identifiers are nucleic acid sequence tags.

44. The method of claim 43, wherein said sequence tags are associated with a moiety that binds said analyte.

45. The method of claim 42, wherein said analyte is a protein.

46. The method of claim 45, wherein said identifiers are peptides.

Abstract

The invention generally relates to methods for maintaining the integrity and identification of a nucleic acid template in a multiplex sequencing reaction. In certain embodiments, methods of the invention involve obtaining a template nucleic acid, incorporating a pair of sequence identifiers into the template, and sequencing the template.

1805923

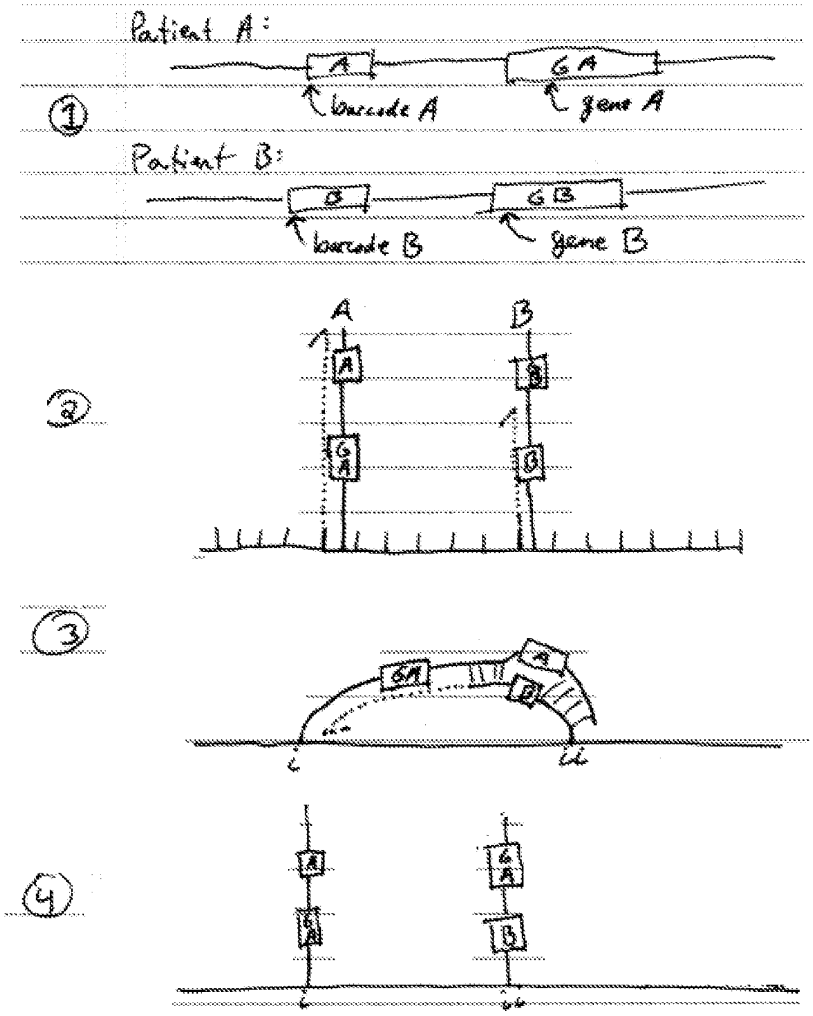

Patient A:
barcode A
gene A
Patient B:
barcode B
gene B
A
B
GA
GB
1
2
3
4

FIGURE 1

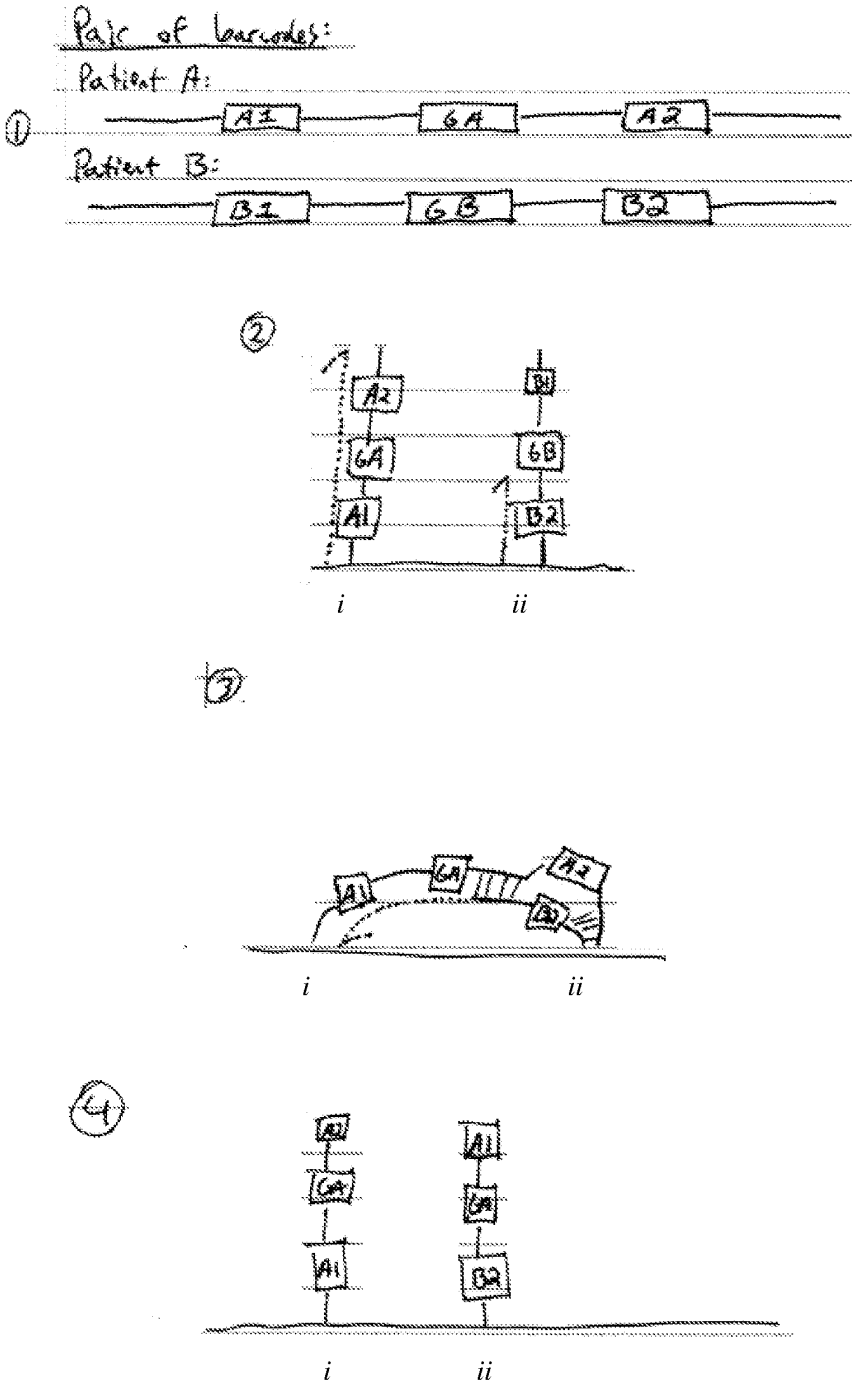

Pair of barcodes:
Patient A:
A1
GA
A2
Patient B:
B1
GB
B2
i
ii
i
ii
i
ii

FIGURE 2

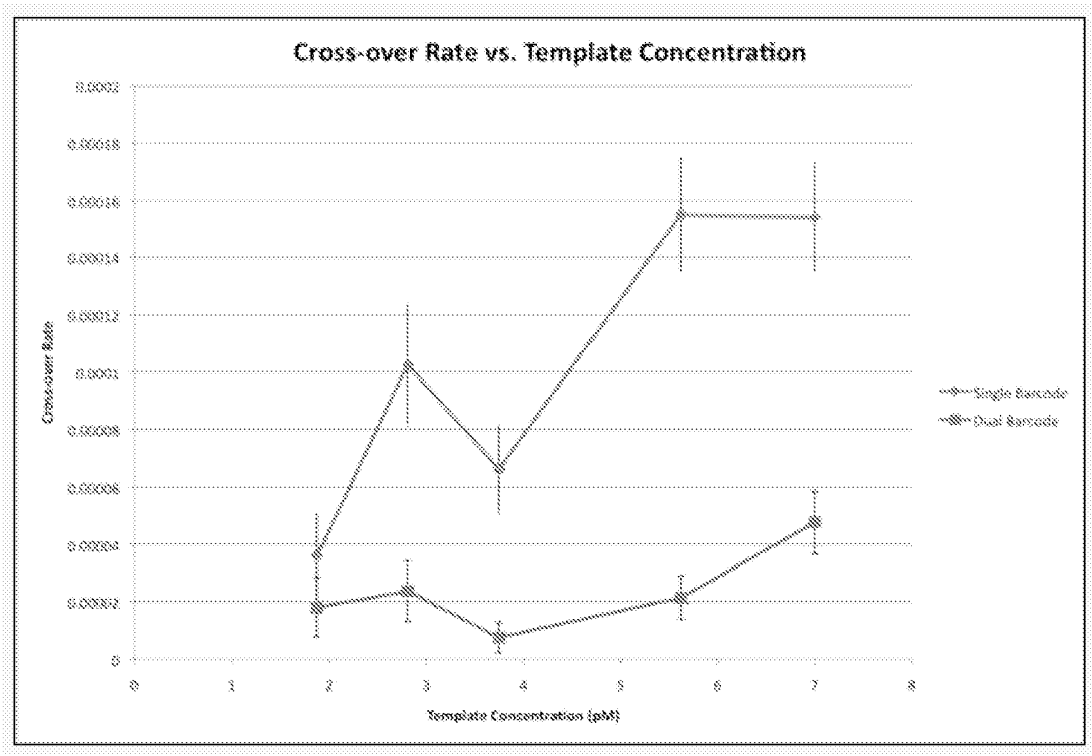

Cross-over Rate vs. Template Concentration
Cross-over Rate
Template Concentration (pM)
Single Barcode
Dual Barcode

FIGURE 3

Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Filer:	Thomas C. Meyers/Franziska Salcedo
Attorney Docket Number:	GSGE-001/01US 29258/14
Filed as Small Entity	

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	82	82
Utility Search Fee	2111	1	270	270
Utility Examination Fee	2311	1	110	110
Pages:				
Claims:				
Claims in excess of 20	2202	26	26	676
Independent claims in excess of 3	2201	1	110	110
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1248**

Electronic Acknowledgement Receipt	
EFS ID:	9828091
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	07-APR-2011
Filing Date:	
Time Stamp:	12:23:23
Application Type:	Utility under 35 USC 111(a)

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Payment Type	Deposit Account
Payment was successfully received in RAM	$1248
RAM confirmation Number	9131
Deposit Account	500369
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Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees) Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees) Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	GSGE-001-01US_SB14_Non-Provisional_ADS.pdf	1800915 250c11b5d6805c42f485df9d352aac98fd0b135e	no	5
Warnings:					
Information:					
2		GSGE_001_01US_Application.pdf	312732 0a841d33252ac892f82433f6504cb00e795ed4c5	yes	38

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	28
	Claims	29	34
	Abstract	35	35
	Drawings-only black and white line drawings	36	38

Warnings:					
Information:					
3	Fee Worksheet (PTO-875)	fee-info.pdf	38373 e7a2ab2da8b1b1942a3b34b59752b779e186bf12	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	2152020		

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
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SCORE Placeholder Sheet for IFW Content

Application Number: 13081660 Document Date: 4/7/2011

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings – Other than Black and White Line Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

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Form Revision Date: February 8, 2006

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/081,660

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	82		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	270		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	110		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	46 minus 20 =	* 26	x 26 =	676	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 110 =	110			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1248		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/081,660	04/07/2011	1634	1248	GSGE-001/01US 29258/14	46	4

CONFIRMATION NO. 4441

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

FILING RECEIPT

OC000000047176555

Date Mailed: 04/18/2011

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Gregory Porreca, Cambridge, MA;
Mark Umbarger, Brookline, MA;
George Church, Brookline, MA;

Assignment For Published Patent Application

GOOD START GENETICS, INC., Boston, MA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/426,817 12/23/2010

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/081,660**

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****

Title

METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

Preliminary Class

435

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where

the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14

CONFIRMATION NO. 4441

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

FORMALITIES LETTER

OC000000047176556

Date Mailed: 04/18/2011

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is missing.
  A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
  Note: If a petition under 37 CFR 1.47 is being filed, an oath or declaration in compliance with 37 CFR 1.63 signed by all available joint inventors, or if no inventor is available by a party with sufficient proprietary interest, is required.

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The drawings must be made on paper that has a white background (see 37 CFR 1.84(e)). For example, drawings on graph paper, lined paper, or paper that has a non-white background are not acceptable. See Figure(s) 1, 2.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- A surcharge (for late submission of filing fee, search fee, examination fee or oath or declaration) as set forth in 37 CFR 1.16(f) of $**65** for a small entity in compliance with 37 CFR 1.27, must be submitted.

SUMMARY OF FEES DUE:

Total fee(s) required within **TWO MONTHS** from the date of this Notice is $**65** for a small entity
- $**65** Surcharge.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/hteffera/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Porreca et al.	ART UNIT:	1634
SERIAL NUMBER:	13/081,660	CONFIRMATION NO.:	4441
FILING DATE:	April 7, 2011	EXAMINER:	NOT YET ASSIGNED
TITLE:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION		

FILED ELECTRONICALLY

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NOTICE TO FILE MISSING PARTS

Dear Sirs:

In response to the Notice to File Missing Parts of Non-provisional Application dated April 18, 2011, Applicants submit herewith replacement drawings and an executed declaration that identifies the application by the above application number and filing date. Applicants hereby authorize the Commissioner to charge any and all fees or credit any overpayment to Deposit Account No. 500369.

Dated: June 15, 2011

Respectfully submitted,
BROWN RUDNICK LLP

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8200
Fax: (617) 856-8201

/s/Thomas Meyers/
Thomas Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

1836038

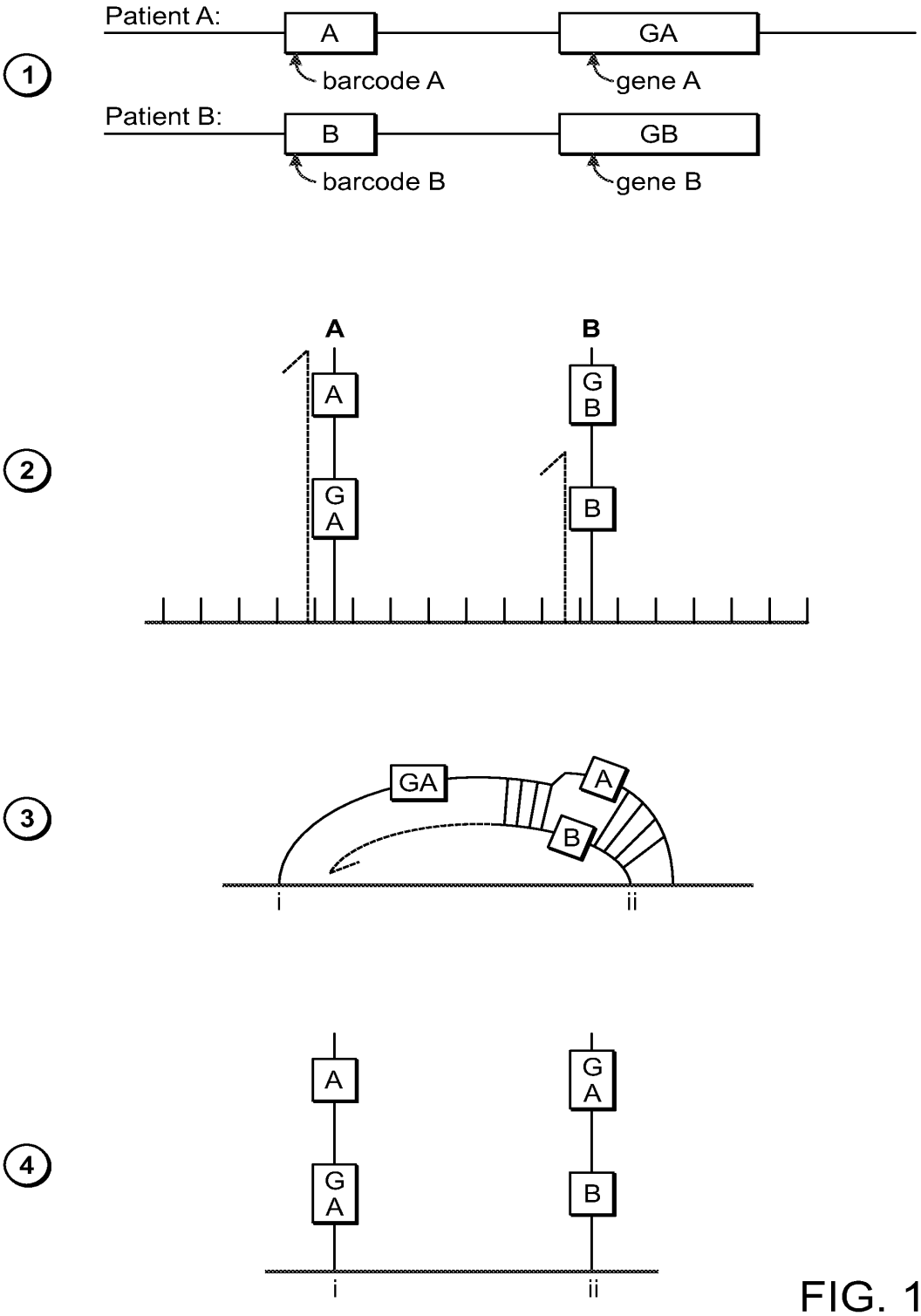

1
Patient A:
A
GA
barcode A
gene A
Patient B:
B
GB
barcode B
gene B
2
A
B
A
GA
GB
B
3
GA
A
B
i
ii
4
A
GA
G
A
B
i
ii

FIG. 1

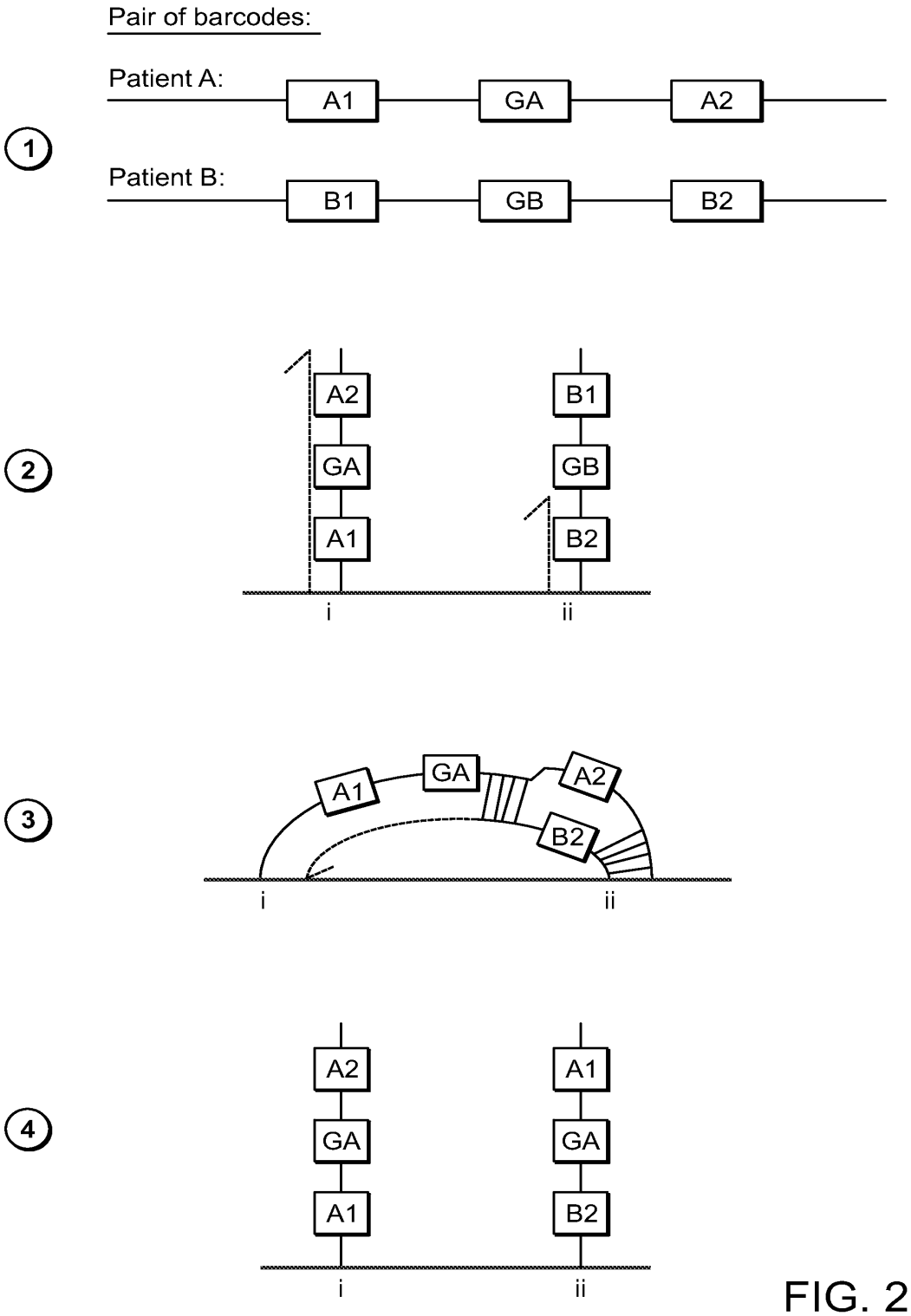

Pair of barcodes:
1
Patient A:
A1
GA
A2
Patient B:
B1
GB
B2
2
A2
GA
A1
B1
GB
B2
i
ii
3
A1
GA
A2
B2
i
ii
4
A2
GA
A1
A1
GA
B2
i
ii

FIG. 2

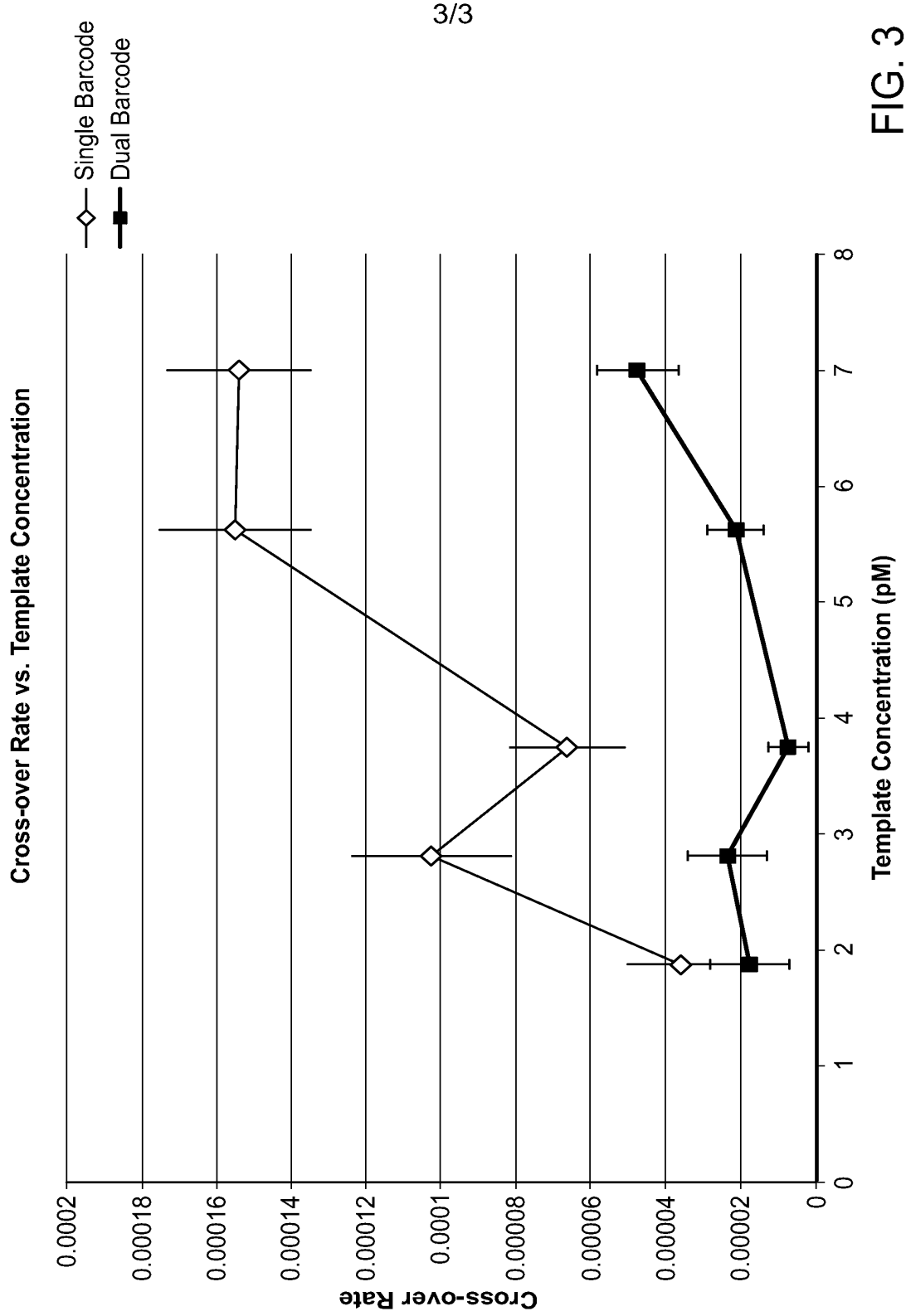

Cross-over Rate vs. Template Concentration
Single Barcode
Dual Barcode
Cross-over Rate
0.0002
0.00018
0.00016
0.00014
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
Template Concentration (pM)
0
1
2
3
4
5
6
7
8

FIG. 3

Attorney Docket No: GSGE-001/01US 29258/14 **PATENT**

DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

the specification of which:

(check one)

[] is attached hereto;

[X] was filed as United States Application Serial No. **13/081,660** on **April 7, 2011**;

[] was filed as PCT International Application No. ___ on ___ and was amended under PCT Article 19 or Article 34 on ___ (if applicable);

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information which is known to me to be material to the patentability of said invention in accordance with 37 C.F.R. §1.56;

I hereby claim foreign priority benefits under 35 U.S.C. §119 and/or §365 of any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America listed below; I have also identified below any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Prior Foreign Application(s)

COUNTRY/INTERNATIONAL	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
			☐ YES ☐ NO
			☐ YES ☐ NO

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

61/426,817 (Application Number) 23 December 2010 (Filing Date) (day, month, year)

(Application Number) (Filing Date) (day, month, year)

I hereby claim the benefit under 35 U.S.C. §120 and/or §365 of any United States application(s) or of any international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date(s) of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Application(s) or PCT International Applications Designating the U.S. for benefit under 35 U.S.C. §120

U.S. APPLICATIONS			STATUS (*check one*)		
U.S. APPLICATION NO.	U.S. FILING DATE (day, month, year)		Pending	Patented	Abandoned
			☐	☐	☐
			☐	☐	☐
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE (day, month, year)	U.S. APPLICATION NOS. (if any)			
			☐	☐	☐
			☐	☐	☐

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of inventor: Gregory Porreca

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 57 Reservoir Street, Cambridge, MA 02138

Full name of inventor: Mark Umbarger

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 5 Winchester Street Unit 101, Brookline, MA 02446

Full name of inventor: George Church

Inventor's signature _____ Date 4/29/11
Residence: US
Citizen of: US
Post Office Address: 218 Kent Street, Brookline, MA 02146

1820642

Attorney Docket No: GSGE-001/01US 29258/14 **PATENT**

DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

the specification of which:

(check one)

[] is attached hereto;

[X] was filed as United States Application Serial No. **13/081,660** on **April 7, 2011**;

[] was filed as PCT International Application No. ___ on ___ and was amended under PCT Article 19 or Article 34 on ___ (if applicable);

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information which is known to me to be material to the patentability of said invention in accordance with 37 C.F.R. §1.56;

I hereby claim foreign priority benefits under 35 U.S.C. §119 and/or §365 of any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America listed below; I have also identified below any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Prior Foreign Application(s)

COUNTRY/INTERNATIONAL	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
			☐ YES ☐ NO
			☐ YES ☐ NO

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

61/426,817 (Application Number) 23 December 2010 (Filing Date) (day, month, year)

(Application Number) (Filing Date) (day, month, year)

I hereby claim the benefit under 35 U.S.C. §120 and/or §365 of any United States application(s) or of any international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date(s) of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Application(s) or PCT International Applications Designating the U.S. for benefit under 35 U.S.C. §120

U.S. APPLICATIONS			STATUS (*check one*)		
U.S. APPLICATION NO.	U.S. FILING DATE (day, month, year)		Pending	Patented	Abandoned
			☐	☐	☐
			☐	☐	☐
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE (day, month, year)	U.S. APPLICATION NOS. (if any)			
			☐	☐	☐
			☐	☐	☐

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of inventor: Gregory Porreca

Inventor's signature _____ Date 4-29-2011
Residence: US
Citizen of: US
Post Office Address: 57 Reservoir Street, Cambridge, MA 02138

Full name of inventor: Mark Umbarger

Inventor's signature _____ Date 4/29/2011
Residence: US
Citizen of: US
Post Office Address: 5 Winchester Street Unit 101, Brookline, MA 02446

Full name of inventor: George Church

Inventor's signature _____ Date _____
Residence: US
Citizen of: US
Post Office Address: 218 Kent Street, Brookline, MA 02146

1820642

Electronic Patent Application Fee Transmittal

Application Number:	13081660
Filing Date:	07-Apr-2011
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Filer:	Thomas C. Meyers/Franziska Salcedo
Attorney Docket Number:	GSGE-001/01US 29258/14

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Late filing fee for oath or declaration	2051	1	65	65
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**65**

Electronic Acknowledgement Receipt	
EFS ID:	10308515
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Franziska Salcedo
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	15-JUN-2011
Filing Date:	07-APR-2011
Time Stamp:	14:25:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$65
RAM confirmation Number	701
Deposit Account	500369
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)	
Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)	
Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	GSGE_001_01US_Response_to_NTFMP.pdf	21245 c39b039e61510c96d469e436d7c2e442a87c69d1	no	1
Warnings:					
Information:					
2	Drawings-only black and white line drawings	GSGE_001_01US_Formal_Drawings.pdf	20169 742517ff2b52e61061ba3ab0e29bb84a84f90cef	no	3
Warnings:					
Information:					
3	Oath or Declaration filed	GSGE_001_01US_Executed_declaration_Church.pdf	111125 7067f5c86e3ab4e846eb373631f5fe9301be9147	no	3
Warnings:					
Information:					
4	Oath or Declaration filed	GSGE_001_01US_Executed_declaration_Porreca_Umbarger.pdf	110212 a3e97b7569fa65d4988d841adb629423051d0c26	no	3
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30499 89275713e8433df64de208948a27ffdfd3b801bd	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	293250		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/081,660

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	82		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	270		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	110		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	46 minus 20 =	* 26	x 26 =	676	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 110 =	110			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1248		TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/081,660	04/07/2011	1634	1313	GSGE-001/01US 29258/14	46	4

CONFIRMATION NO. 4441

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

UPDATED FILING RECEIPT



Date Mailed: 06/23/2011

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Gregory Porreca, Cambridge, MA;
Mark Umbarger, Brookline, MA;
George Church, Brookline, MA;

Assignment For Published Patent Application

GOOD START GENETICS, INC., Boston, MA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/426,817 12/23/2010

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

If Required, Foreign Filing License Granted: 04/14/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/081,660**

Projected Publication Date: 06/28/2012

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****

Title

METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

Preliminary Class

435

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where

the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	PORRECA, Gregory
	Art Unit	1637
	Examiner Name	STRZELECKA, Teresa
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					
If you wish to add additional U.S. Patent citation information please click the Add button.						

U.S.PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20080269068		2008-10-30	Church	
	2	20100035243		2010-02-11	Muller	
	3	20100297626		2010-11-25	McKernan	
If you wish to add additional U.S. Published Application citation information please click the Add button.						

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	PORRECA, Gregory
	Art Unit	1637
	Examiner Name	STRZELECKA, Teresa
	Attorney Docket Number	GSGE-001/01US 29258/14

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	International Search Report and Written Opinion in corresponding international application PCT/US2011/065098, filed 15 December 2011, mailed 03 April 2012 (10 pages)	☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	PORRECA, Gregory
	Art Unit	1637
	Examiner Name	STRZELECKA, Teresa
	Attorney Docket Number	GSGE-001/01US 29258/14

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/s/Thomas C. Meyers/	Date (YYYY-MM-DD)	2012-05-24
Name/Print	Thomas C. Meyers	Registration Number	36989

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

TCM|AMS 29258|28WO

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)*
Applicant's or agent's file reference 29258/28WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 11/65098	International filing date *(day/month/year)* 15 December 2011 (15.12.2011)
Applicant GOOD START GENETICS, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 03 APR 2012
Applicant's or agent's file reference 29258/28WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 11/65098	International filing date *(day/month/year)* 15 December 2011 (15.12.2011)
Applicant GOOD START GENETICS, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 29258/28WO	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 11/65098	International filing date *(day/month/year)* 15 December 2011 (15.12.2011)	(Earliest) Priority Date *(day/month/year)* 23 December 2010 (23.12.2010)
Applicant GOOD START GENETICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☒ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/65098

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/00; C12Q 1/68 (2012.01)
USPC - 536/23.1, 435/6.11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C07H 21/00; C12Q 1/68 (2012.01)
USPC: 536/23.1, 435/6.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 536/24.3, 435/6.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, Google Scholar: Sequence identifier$2, 5', 3', sequenc$6, template$2, nucleic acid$2, barcode, validat$4, amplif$4, target polynucleotide$2, sequence identif$6, tag$2, detect$4, label$2, validate$4, identif$4, detect$4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---------- Y	US 2010/0297626 A1 (MCKERNAN et al.) 25 November 2010 (25.11.2010); abstract; para [0026], [0027], [0028], [0029], [0075], [0087], [0096], [0110], [0116], [0117], [0127], [0141], [0142]. [0144], [0163]. [0215], [0323], [0332], [0336], [0337], [0342],	1-2, 4-15 ---------- 3, 16-46
Y	US 2008/0269068 A1 (CHURCH et al.) 30 October 2008 (30.10.2008); para [0007], [0022]	3, 16-41
Y	US 2010/0035243 A1 (MULLER et al.) 11 February 2010 (11.02.2010); para [0012], [0017]	42-46

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2012 (14.03.2012)	03 APR 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	03 APR 2012

Applicant's or agent's file reference	FOR FURTHER ACTION
29258/28WO	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 11/65098	15 December 2011 (15.12.2011)	23 December 2010 (23.12.2010)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C07H 21/00; C12Q 1/68 (2012.01)
USPC - 536/23.1, 435/6.11

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	14 March 2012 (14.03.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/65098

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/65098

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	3, 11-12, 16-46	YES
	Claims	1-2, 4-10, 13-15	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-46	NO
Industrial applicability (IA)	Claims	1-46	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-2, 4-10 and 13-15 lack novelty under PCT Article 33(2) as being anticipated by US 2010/0297626 A1 to McKernan et al. (hereinafter 'McKernan').

Regarding claim 1, McKernan teaches a method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction (abstract, para [0087]), the method comprising:
obtaining a template nucleic acid (abstract);
incorporating at least two sequence identifiers into the template (abstract); and
sequencing the template (abstract).

Regarding claim 2 McKernan further teaches the method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template (para [0026], [0027]).

Regarding claim 4, McKernan further teaches the method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template (para [0026]).

Regarding claim 5, McKernan further teaches the method according to claim 2, wherein the first and second identifiers are the same (para [0144]- paired tags have the same sequence).

Regarding claim 6, McKernan further teaches the method according to claim 2, wherein the first and second identifiers are different (para [0163]).

Regarding claim 7, McKernan further teaches the method according to claim 1, wherein prior to sequencing, the template is attached to a substrate (para [0332]).

Regarding claim 8, McKernan further teaches the method according to claim 2, wherein the template is directly attached to the substrate (para [0336]).

Regarding claim 9, McKernan further teaches the method according to claim 2, wherein the template is indirectly attached to the substrate (para [0336]).

Regarding claim 10, McKernan further teaches the method according to claim 7, further comprising, amplifying the template (para [0075]).

Regarding claim 13, McKernan further teaches the method according to claim 1, wherein sequencing comprises:
hybridizing a primer to the template to form a template/primer duplex (para [0215]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0215], [0116]);
detecting a signal from the incorporated labeled nucleotide (para [0197]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid (para [0323]).

Regarding claim 14, McKernan further teaches the method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0029]).

Regarding claim 15, McKernan further teaches the method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides (para [0029]).

Claims 11-12 and lacks inventive step under PCT Article 33(3) as being obvious over McKernan.

Regarding claim 11, McKernan teaches the method according to claim 1, and further makes obvious wherein sequencing is sequencing by synthesis (para [0127], sequencing by synthesis is found to lead to errors, however the possibility of sequencing by synthesis is identified and made obvious as a possible sequencing method).

*****Continued in Supplemental Box*****

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V(2):

Regarding claim 12, McKernan makes obvious the method according to claim 11, and further teaches wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0117]).

Claims 3, 16-41 lack inventive step under PCT Article 33(3) as being obvious over McKernan in view of US 2008/0269068 A1 to Church et al. (hereinafter 'Church').

Regarding claim 3, McKernan teaches the method according to claim 2, however fails to teach wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence. McKernan does teach wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first identifier sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second identifier sequence; and a fourth known sequence (para [0027], [0096], [0110]). McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 16, McKernan teaches the method according to claim 1, however fails to teach wherein the pair of identifiers are first and second barcode sequences that are attached to the template. McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 17, the combination of McKernan and Church makes obvious the method according to claim 16, and McKernan further makes obvious wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template (para [0141]-[0142], if barcodes are being utilized as the identifies as made obvious in claim 16, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 18, the combination of McKernan and Church makes obvious the method according to claim 17, and McKernan further teaches wherein the first and second barcode sequences are the same (para [0144], same sequence).

Regarding claim 19, the combination of McKernan and Church makes obvious the method according to claim 17, and McKernan further teaches wherein the first and second barcode sequences are different (para [0163]).

Regarding claim 20, McKernan teaches a method for analyzing a nucleic acid (abstract), the method comprising:
obtaining a nucleic acid template (abstract);however fails to teach
attaching a pair of barcode sequences to the template; and McKernan further teaches
sequencing the template (abstract).
McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 21, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template (para [0026]).

Regarding claim 22, the combination of McKernan and Church makes obvious the method according to claim 21, and McKernan makes obvious wherein after attaching of the pair of barcode sequences,
the template has the following configuration from 5' to 3': a first known sequence; a first
barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence (para [0026]-[0027], if barcodes are being utilized as the identifies as made obvious in claim 16, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 23, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template (para [0026]-[0027]).

Regarding claim 24, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein prior to sequencing, the template is attached to a substrate (para [0332]).

Regarding claim 25, the combination of McKernan and Church makes obvious the method according to claim 24, and McKernan further teaches wherein the template is directly attached to the substrate (para [0336]).

Regarding claim 26, the combination of McKernan and Church makes obvious the method according to claim 24, and McKernan further teaches wherein the template is indirectly attached to the substrate (para [0336]).

******Continued in Supplemental Box******

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box:

Regarding claim 27, the combination of McKernan and Church makes obvious the method according to claim 24, and McKernan further teaches the method further comprising, amplifying the template (para [0075]).

Regarding claim 28, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further makes obvious wherein sequencing is sequencing by synthesis (para [0127], sequencing by synthesis is found to lead to errors, however the possibility of sequencing by synthesis is identified and made obvious as a possible sequencing method).

Regarding claim 29, the combination of McKernan and Church makes obvious the method according to claim 28, and McKernan further makes obvious wherein the sequencing by synthesis is single molecule sequencing by synthesis.

Regarding claim 30, the combination of McKernan and Church makes obvious the method according to claim 20, and McKernan further teaches wherein sequencing comprises:
hybridizing a primer to the template to form a template/primer duplex (para [0215]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably
labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0215], [0116]);
detecting a signal from the incorporated labeled nucleotide (para [0323]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid (para [0117]).

Regarding claim 31, the combination of McKernan and Church makes obvious the method according to claim 29, and McKernan further teaches wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0029]).

Regarding claim 32, the combination of McKernan and Church makes obvious the method according to claim 31, and McKernan further teaches wherein the optically labeled nucleotide is a fluorescently labeled nucleotides (para [0029]).

Regarding claim 33, the combination of McKernan and Church makes obvious the method according to claim 23, and McKernan further teaches wherein the first and second barcode sequences are the same (para [0144]- have the same sequence. If barcodes are being utilized as the identifies as made obvious in claim 23, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 34, the combination of McKernan and Church makes obvious the method according to claim 23, and McKernan further teaches wherein the first and second barcode sequences are different (para [0163]).

Regarding claim 35, McKernan teaches a method for analyzing a nucleic acid (abstract), the method comprising:
obtaining a nucleic acid template (abstract); however fails to teach
attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template;
McKernan goes on to teach
amplifying the template (para [0075]); and
sequencing the template (abstract).
McKernan does teach attaching an identifier sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (para [0141]-[0142]).
McKernan does teach wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]), however fails to teach wherein the first and second identifiers are barcode sequences. Church teaches a barcode used as a tag attached to a nucleic acid attached to a substrate (para [0007], [0022]) and thus it would have been obvious to one of ordinary skill in the art to use barcodes as the first and second identifiers on the nucleic acid taught by McKernan in order to increase the accuracy of the method.

Regarding claim 36, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further makes obvious wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence (para [0026]-[0027], If barcodes are being utilized as the identifies as made obvious in claim 23, it would have been obvious to one of ordinary skill in the art to use them in the same manner that the tags were utilized by McKernan).

Regarding claim 37, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template (para [0141]-[0142]).

Regarding claim 38, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein prior to amplifying, the template is immobilized to a solid support (para [0332])

Regarding claim 39, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein the first and second barcode sequences are the same (para [0144], tags have the same sequence).

Regarding claim 40, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein the first and second barcode sequences are different (para [0163]).
*****Continued in Supplemental Box*****

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Previous Supplemental Box:

Regarding claim 41, the combination of McKernan and Church makes obvious the method according to claim 35, and McKernan further teaches wherein sequencing comprises:
hybridizing a primer to the template to form a template/primer duplex (para [0215]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably
labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0215], [0116]);
detecting a signal from the incorporated labeled nucleotide (para [0323]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential
detection of incorporated labeled nucleotide determines the sequence of the nucleic acid (para [0323]).

Claims 42-46 lack inventive step under PCT Article 33(3) as being obvious over McKernan in view of US 2010/0035243 A1 to Muller et al. (hereinafter 'Muller').

Regarding claim 42, concerning a method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte. McKernan does teach a method for validating detection of a nucleic acid sequence, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said sequence (abstract). McKernan further teaches wherein samples can be barcoded to maintain integrity of sample tracking (para [0342]). Muller teaches using a specific barcode sequence for detection of analyte (para [0017]). It would have been thus obvious to one of ordinary skill in the art to utilize the method taught by McKernan to identify an analytein order to increase the accuracy of the method.

Regarding claim 43, the combination of McKernan and Muller makes obvious the method of claim 42, and McKernan further teaches wherein said identifiers are nucleic acid sequence tags (abstract).

Regarding claim 44. the combination of McKernan and Muller makes obvious the method of claim 43, however fails to teach wherein said sequence tags are associated with a moiety that binds said analyte. Muller teaches using a specific barcode sequence for detection of analyte (para [0017]). It would have been thus obvious to one of ordinary skill in the art to utilize the method taught by McKernan to identify an analyte in order to increase the accuracy of the method.

Regarding claim 45, the combination of McKernan and Muller makes obvious the method of claim 42, and Muller further teaches wherein said analyte is a protein (para [0012]).

Regarding claim 46, the combination of McKernan and Muller makes obvious the method of claim 45,and Muller further teaches wherein said identifiers are peptides (para [0047]).

Claims 1-46 have industrial applicability under PCT Article 33(4) because the subject matter can be made or used in industry.

Electronic Acknowledgement Receipt	
EFS ID:	12854214
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Michelle Aiello
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	24-MAY-2012
Filing Date:	07-APR-2011
Time Stamp:	12:54:01
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	GSGE-001-01US-IDS-052412.pdf	61797 e8922ac76fc0088b00cb6ec3c891d1e1af36b112	no	3
Warnings:					
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2	Non Patent Literature	ISR-Writ-Op-PCT-US2011-065098.pdf	639905 9f89420474cc192c936013e59780eba897c23eaa	no	10
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			Total Files Size (in bytes):	701702	

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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14

CONFIRMATION NO. 4441

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PUBLICATION NOTICE



Title:METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

Publication No.US-2012-0164630-A1
Publication Date:06/28/2012

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The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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PTO/SB/08a (01-10)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	13/081,660 - Conf. No. 4441
	Filing Date	04/07/2011
	First Named Inventor	Gregory PORRECA
	Art Unit	1634
	Examiner Name	
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6,403,320	B1	06-2002	Read et al.	
If you wish to add additional U.S. Patent citation information please click the Add button.						

U.S.PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	2009/0163366	A1	06-2009	Nickerson et al.	
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FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	WO 2009/036525	WO	A2	03/26/2009	CUPPENS, Harry		☐
If you wish to add additional Foreign Patent Document citation information please click the Add button								

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13/081,660 - Conf. No. 4441
	Filing Date	04/07/2011
	First Named Inventor	Gregory PORRECA
	Art Unit	1634
	Examiner Name	
	Attorney Docket Number	GSGE-001/01US 29258/14

			☐

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13/081,660 - Conf. No. 4441
	Filing Date	04/07/2011
	First Named Inventor	Gregory PORRECA
	Art Unit	1634
	Examiner Name	
	Attorney Docket Number	GSGE-001/01US 29258/14

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2012-08-06
Name/Print	Thomas C. Meyers	Registration Number	36,989

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	13427586
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Thomas Hellenthal
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	06-AUG-2012
Filing Date:	07-APR-2011
Time Stamp:	16:44:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	Cuppens_WO2009036525A2.PDF	911342 569ee1f4c4efce5a5d27a004eaeb4a99d2d9723f	no	32
Warnings:					
Information:					

2	Information Disclosure Statement (IDS) Form (SB08)	GSGE_001_01US_Supplemental_IDS.pdf	1937731 6962a7574d5d152fcab91011e33b16ba59995025	no	4

Warnings:	
Information:	
This is not an USPTO supplied IDS fillable form	
Total Files Size (in bytes):	2849073

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

21710 7590 04/18/2013
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
STRZELECKA, TERESA E	
ART UNIT	PAPER NUMBER
1637	
MAIL DATE	DELIVERY MODE
04/18/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 13/081,660	Applicant(s) PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *1* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-46* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *1-46* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some * c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Interim copies:

a)☐ All b)☐ Some c)☐ None of the: Interim copies of the priority documents have been received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-41, drawn to a method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction, the method comprising: obtaining a template nucleic acid; incorporating at least two sequence identifiers into the template; and sequencing the template, classified in class 435, subclass 6.1, for example.

II. Claims 42-46, drawn to a method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte, classified in class 435, subclass 7.1, for example.

The inventions are distinct, each from the other because of the following reasons:

2. Inventions I and II are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions are drawn to methods with different goals, method steps and materials.

The instant specification does not disclose that these methods would be used together. The method of validating an integrity of nucleic acid template using nucleic acid barcodes (group I) and the method of validating detection of any analyte (group II) are unrelated as they comprise distinct steps and utilize different products which demonstrates that each method has a different mode of operation. Each invention performs this function using a structurally and functionally

divergent material. Therefore, each method is divergent in materials and steps. For these reasons the Inventions I and II are patentably distinct. Furthermore, the distinct steps and products require separate and distinct searches. The inventions of Groups I and II have a separate status in the art as shown by their different classifications. As such, it would be burdensome to search the inventions of Groups I and II together.

3. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because at least the following reason(s) apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention**.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

4. This application contains claims directed to the following patentably distinct species:

Group I

Species of identifier position

A) a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template (claims 2, 3, 21, 22, 35, 36),

B) a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template (claims 4, 17, 23, 37).

Species of identifiers

C) first and second identifiers are the same (claims 5, 18, 33, 39),

D) first and second identifiers are different (claims 6, 19, 34, 40).

Species of template attachment

E) template is directly attached to a substrate (claims 8, 25),

F) template is indirectly attached to a substrate (claims 9, 26).

5. The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species from each of the sets of species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1, 20 and 35 are generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or a grouping of patentably indistinct species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**,

including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

6. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the

application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TERESA E STRZELECKA
Primary Examiner
Art Unit 1637

/TERESA E STRZELECKA/
Primary Examiner, Art Unit 1637
April 17, 2013

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Porreca et al.	ART UNIT:	1637
SERIAL NUMBER:	13/081,660	CONFIRMATION NO.:	4441
FILING DATE:	April 7, 2011	EXAMINER:	Strzelecka, Teresa E.
TITLE:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION		

FILED ELECTRONICALLY

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO RESTRICTION REQUIREMENT

This paper is in response to a Restriction Requirement mailed April 18, 2012 from the United States Patent and Trademark Office. Applicants authorize any and all required fees due with this response to be charged to Deposit Account No. 500369.

Amendments to the specification begin on page 2.

Amendments to the claims begin on page 3.

Remarks begin on page 10.

Amendments to the specification

No amendments to the specification are made herein.

Amendment to the claims

Please withdraw claims 4, 6, 9, 17, 19, 23, 26, 34, 37, 40, 42-46 as follows. This list of claims replaces all prior versions and listings of claims in this application.

1. (Original) A method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction, the method comprising:

obtaining a template nucleic acid;

incorporating at least two sequence identifiers into the template; and

sequencing the template.

2. (Original) The method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

3. (Original) The method according to claim 2, wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

4. (Withdrawn) The method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

5. (Original) The method according to claim 2, wherein the first and second identifiers are the same.

6. (Withdrawn) The method according to claim 2, wherein the first and second identifiers are different.

7. (Original) The method according to claim 1, wherein prior to sequencing, the template is attached to a substrate.

8. (Original) The method according to claim 2, wherein the template is directly attached to the substrate.

9. (Withdrawn) The method according to claim 2, wherein the template is indirectly attached to the substrate.

10. (Original) The method according to claim 7, further comprising, amplifying the template.

11. (Original) The method according to claim 1, wherein sequencing is sequencing by synthesis.

12. (Original) The method according to claim 11, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

13. (Original) The method according to claim 1, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

14. (Original) The method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

15. (Original) The method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

16. (Original) The method according to claim 1, wherein the pair of identifiers are first and second barcode sequences that are attached to the template.

17. (Withdrawn) The method according to claim 16, wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template.

18. (Original) The method according to claim 17, wherein the first and second barcode sequences are the same.

19. (Withdrawn) The method according to claim 17, wherein the first and second barcode sequences are different.

20. (Original) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a pair of barcode sequences to the template; and

sequencing the template.

21. (Original) The method according to claim 20, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

22. (Original) The method according to claim 21, wherein after attaching of the pair of barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

23. (Withdrawn) The method according to claim 20, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

24. (Original) The method according to claim 20, wherein prior to sequencing, the template is attached to a substrate.

25. (Original) The method according to claim 24, wherein the template is directly attached to the substrate.

26. (Withdrawn) The method according to claim 24, wherein the template is indirectly attached to the substrate.

27. (Original) The method according to claim 24, further comprising, amplifying the template.

28. (Original) The method according to claim 20, wherein sequencing is sequencing by synthesis.

29. (Original) The method according to claim 28, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

30. (Original) The method according to claim 20, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

31. (Original) The method according to claim 29, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

32. (Original) The method according to claim 31, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

33. (Original) The method according to claim 23, wherein the first and second barcode sequences are the same.

34. (Withdrawn) The method according to claim 23, wherein the first and second barcode sequences are different.

35. (Original) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template;

amplifying the template; and

sequencing the template.

36. (Original) The method according to claim 35, wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

37. (Withdrawn) The method according to claim 35, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

38. (Original) The method according to claim 35, wherein prior to amplifying, the template is immobilized to a solid support.

39. (Original) The method according to claim 35, wherein the first and second barcode sequences are the same.

40. (Withdrawn) The method according to claim 35, wherein the first and second barcode sequences are different.

41. (Original) The method according to claim 35, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

42. (Withdrawn) A method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte.

43. (Withdrawn) The method of claim 42, wherein said identifiers are nucleic acid sequence tags.

44. (Withdrawn) The method of claim 43, wherein said sequence tags are associated with a moiety that binds said analyte.

45. (Withdrawn) The method of claim 42, wherein said analyte is a protein.

46. (Withdrawn) The method of claim 45, wherein said identifiers are peptides.

Remarks

In response to the Restriction Requirement mailed April 18, 2013, Applicants elect Group I (claims 1-41) drawn to methods for validating the integrity of a nucleic acid template in a multiplex sequencing reaction. Applicants additionally elect the following species: a first identifier incorporated into a 5' portion of the template and a second identifier incorporated into a 3' portion of the template (claims 2, 3, 21, 22, 35, 36); first and second identifiers being the same (claims 5, 18, 33, and 39); and template is directly attached to a substrate (claims 8 and 25). Accordingly, claims 4, 6, 9, 17, 19, 23, 26, 34, 37, 40, 42-46 are here withdrawn.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: April 30, 3013

Respectfully submitted,
BROWN RUDNICK LLP

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

61150918

Electronic Acknowledgement Receipt	
EFS ID:	15646327
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Thomas Hellenthal
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	30-APR-2013
Filing Date:	07-APR-2011
Time Stamp:	11:18:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		GSGE_001_01US_Response_to_restriction_requirement.pdf	39750 294cceca1daca5bd039f3ce3e0d60e11f7baf4a9	yes	10

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response to Election / Restriction Filed	1	1
	Specification	2	2
	Claims	3	9
	Applicant Arguments/Remarks Made in an Amendment	10	10
Warnings:			
Information:			
	Total Files Size (in bytes):	39750	

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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/081,660	Filing Date 04/07/2011	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 04/30/2013	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 46	Minus	** 46	= 0	X $40 =	0
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $210 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/REGINA BALTIMORE/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	13081660
Filing Date	2011-04-07
First Named Inventor	Gregory Porreca
Art Unit	1637
Examiner Name	T. E. Strzelecka
Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20100330619		2010-12-30	Willis et al.	
	2	20080269068		2008-10-30	CHURCH et al.	

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

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NON-PATENT LITERATURE DOCUMENTS Remove

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	International Search Report and Written Opinion for PCT/US12/29790, mailed June 14, 2012 17 pages	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2013-05-06
Name/Print	Thomas C. Meyers	Registration Number	36989

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal	
Application Number:	13081660
Filing Date:	07-Apr-2011
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Filer:	Thomas C. Meyers/Kelley Froehlich
Attorney Docket Number:	GSGE-001/01US 29258/14
Filed as Small Entity	

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	2806	1	90	90
		Total in USD ($)		**90**

Electronic Acknowledgement Receipt	
EFS ID:	15666287
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Kelley Froehlich
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	06-MAY-2013
Filing Date:	07-APR-2011
Time Stamp:	17:47:27
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$90
RAM confirmation Number	5574
Deposit Account	500369
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	61152009_1-ISR-Written_Opinion_1.PDF	1119306 09411fd2c38df41a8f87f2b2b57027148cec4c68	no	17
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	GSGE-001-01US.pdf	612482 cfd1fe6673ba4345f52a89f1ab83239b92056a34	no	4
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30694 7d20cfade36bba8449c02478edc2b608c10ec792	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1762482		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

TCM/AMS 29258/31 WO

PATENT COOPERATION TREATY

NOTED ON SYSTEM

From the INTERNATIONAL SEARCHING AUTHORITY

To:
THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference GSGE-006/01WO 29258/31	Date of mailing *(day/month/year)* **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 12/29790	International filing date *(day/month/year)* 20 March 2012 (20.03.2012)
Applicant GOOD START GENETICS, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 14 JUN 2012
Applicant's or agent's file reference GSGE-006/01WO 29258/31	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 12/29790	International filing date *(day/month/year)* 20 March 2012 (20.03.2012)
Applicant GOOD START GENETICS, INC.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Authorized officer Lee W. Young
Facsimile No. 571-273-3201	Telephone No. PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GSGE-006/01WO 29258/31	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/29790	International filing date *(day/month/year)* 20 March 2012 (20.03.2012)	(Earliest) Priority Date *(day/month/year)* 31 March 2011 (31.03.2011)
Applicant GOOD START GENETICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☐ the text is approved as submitted by the applicant.

   ☒ the text has been established by this Authority to read as follows:

IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☒ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/29790

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C07H 21/04 (2012.01)
USPC - 435/6.1, 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/68; C07H 21/04 (2012.01)
USPC: 435/6.1, 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.11, 436/94, 536/22.1, 536/24.3, 536/24.33

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, Google Scholar: Barcode, template, identifier, multiplex sequencing, identical, tag, validate, solution, nucleic acid, synthesis, substrate, contact, detect, polymerase, duplex, quantification, contamination, oligonucleotide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0330619 A1 (WILLIS et al.) 30 December 2010 (30.12.2010); para [0012], [0014], [0094], [0131], [0155], [0199], [0228]-[0229], [0273], [0279], [0286], [0294], [0297], [0355], [0365]	1-21
Y		22-33
Y	US 2008/0269068 A1 (CHURCH et al.) 30 October 2008 (30.10.2008); para [0009]	22-33

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2012 (25.05.2012)	14 JUN 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	14 JUN 2012

Applicant's or agent's file reference	FOR FURTHER ACTION
GSGE-006/01WO 29258/31	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/29790	20 March 2012 (20.03.2012)	31 March 2011 (31.03.2011)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68; C07H 21/04 (2012.01)
USPC - 435/6.1, 536/23.1

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	25 May 2012 (25.05.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/29790

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/29790

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	22-33	YES
	Claims	1-21	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-33	NO
Industrial applicability (IA)	Claims	1-33	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-21 lack novelty under PCT Article 33(2) as being anticipated by US 2010/0330619 A1 Willis et al. (hereinafter Willis).

Regarding claim 1, concerning a method for validating the integrity of a nucleic acid template Willis teaches the method comprising:
obtaining a solution comprising a template nucleic acid (para [0012], [0101], [0114]);
introducing an identifier nucleic acid to the solution (para [0012], attach identifiable marker);
incorporating an identical barcode oligonucleotide into the template and the identifier nucleic acids (para [0131]); and
sequencing the template and the identifier nucleic acids (para [0283], [0284]).

Regarding claim 2, Willis further teaches the method according to claim 1, wherein prior to sequencing, the template and the identifier are attached to a substrate (para [0286], [0294]).

Regarding claim 3, Willis further teaches the method according to claim 2, wherein the template and the identifier are directly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 4, Willis further teaches the method according to claim 2, wherein the template and the identifier are indirectly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 5, Willis further teaches the method according to claim 1, further comprising, amplifying the template and the identifier (para [0014], [0138])

Regarding claim 6, Willis further teaches the method according to claim 1, wherein sequencing is sequencing by synthesis (para [0218], [0165], [0202]).

Regarding claim 7, Wills further teaches the method according to claim 6, wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0365]).

Regarding claim 8, Willis further teaches the method according to claim 1, wherein sequencing comprises: hybridizing a primer to both the template and the identifier to form a template/primer duplex and an identifier/primer duplex (para [0012], [0138]);
contacting both duplexes with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primers of both duplexes in a template-dependent manner (para [0228]-[0229]; [0138]);
detecting a signal from the incorporated labeled nucleotide (para [0225]-[0226]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the template nucleic acid and the identifier nucleic acid (para [0225]-[0226]).

Regarding claim 9, Willis further teaches the method according to claim 8, wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0199]).

Regarding claim 10, Willis further teaches the method according to claim 9, wherein the optically labeled nucleotide is a fluorescently labeled nucleotide (para [0199]).

*****Continued in Supplemental Box*****

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/29790

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fu lly supported by the description, are made:
Claims 1-10 are objected to as being unclear as to the term "integrity". The claims fail to clearly set forth the technical features of the invention for which protection is sought, as according to PCT Rule 6. Claim 1 is generally narrative and indefinite with regard to the tem "integrity". Specifically, the claim fail to define the matter for which protection is sought with sufficient detail such that the claims are not clear and consise. Further, the specifications fails to enable one of ordinary skill in the art to practice the claims because the term is not defined nor fully supported.

Form PCT/ISA/237 (Box No. VIII) (July 2011)

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V(2):

Regarding claim 11 concerning a method for quantifying a contamination rate in a sequencing reaction, Willis teaches the method comprising:
determining a number of barcode reads in a sequencing reaction that are correctly correlated with an identifier nucleic acid (para [0012], [0131]), wherein the identifier nucleic acid is associated with a template nucleic acid (para [0012]), and the barcode reads are generated by a sequencing apparatus (para [0094]);
determining a number of barcode reads in the sequencing reaction that are incorrectly correlated with the identifier nucleic acid (para [0297]-[0299]); and
obtaining a fraction of the two numbers, thereby quantifying a contamination rate in the sequencing reaction (para [0298]).

Regarding claim 12, Willis further teaches the method according to claim 11, wherein determining comprises:
introducing an identifier nucleic acid to a solution (para [0012], attach identifiable marker) comprising a template nucleic acid (para [0131]);
incorporating an identical barcode oligonucleotide into the template and the identifier nucleic acids (para [0131]); and
sequencing the template and the identifier nucleic acids (para [0283]).

Regarding claim 13, Willis further teaches the method according to claim 12, wherein prior to sequencing, the template and the identifier are attached to a substrate (para [0286], [0294]).

Regarding claim 14, Willis further teaches the method according to claim 13, wherein the template and the identifier are directly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 15, Willis further teaches the method according to claim 13, wherein the template and the identifier are indirectly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 16, Willis further teaches the method according to claim 12, further comprising, amplifying the template and the identifier (para [0014], [0138])

Regarding claim 17, Willis further teaches the method according to claim 12, wherein sequencing is sequencing by synthesis (para [0218], [0165], [0202]).

Regarding claim 18, Wills further teaches the method according to claim 17, wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0365]).

Regarding claim 19, Willis further teaches the method according to claim 12, wherein sequencing comprises: hybridizing a primer to both the template and the identifier to form a template/primer duplex and an identifier/primer duplex (para [0012], [0138]);
contacting both duplexes with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primers of both duplexes in a template-dependent manner (para [0228]-[0229]; [0138]);
detecting a signal from the incorporated labeled nucleotide (para [0225]-[0226]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the template nucleic acid and the identifier nucleic acid (para [0225]-[0226]).

Regarding claim 20, Willis further teaches the method according to claim 19, wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0199

Regarding claim 21, Willis further teaches the method according to claim 20, wherein the optically labeled nucleotide is a fluorescently labeled nucleotide (para [0199]).

Claims 22-33 lack inventive step under PCT Article 33(3) as being obvious over Willis in view of 2008/0269068 A1 to Church et al. (hereinafter .Church.).

Regarding claim 22, concerning a method for identifying a contamination in a batch of barcode oligonucleotides, Willis teaches the method comprising:
preparing a plurality of batches of barcoded identifier nucleic acids, wherein each batch comprises a unique identifier nucleic acid attached to a unique barcode oligonucleotide (para [0131]); however fails to teach
pooling the batches;
sequencing the pooled batches;
and identifying barcode oligonucleotides that are improperly paired with identifier nucleic acids.
Church teaches a method of identifying a contamination in a batch of barcode oligonucleotides (para [0009], detect that barcodes have been correctly attached and thus also being able to detect incorrect attachment)
pooling the batches (para [0009]);
sequencing the pooled batches (para [0009]);
and identifying barcode oligonucleotides that are improperly paired with identifier nucleic acids (para [0009], detect that barcodes have been correctly attached and thus also being able to detect incorrect attachment). In light of the fact that Church teaches a method of confirming correct barcode attachment to an oligonucleotide attached to an identifier (para [0009]), it would have been obvious to one of ordinary skill in the art to confirm correct attachment of barcodes to the sequence of interest in order to further insure the validity of the results found in the method taught by Willis.

*****Continued in Supplemental Box*****

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
*****Continuation of Previous Supplemental Box*****

Regarding claim 23, the combination of Willis and Church makes obvious the method according to claim 22, and Willis further teaches wherein attaching comprises a PCR reaction (para [0146]).

Regarding claim 24, the combination of Willis and Church makes obvious the method according to claim 22, and Wills further teaches wherein attaching comprises a ligation reaction (para [0381]).

Regarding claim 25, the combination of Willis and Church makes obvious the method according to claim 22, and Willis further teaches wherein prior to sequencing, the barcoded identifier is attached to a substrate (para [0286], [0294]).

Regarding claim 26, the combination of Willis and Church makes obvious the method according to claim 25, and Willis further teaches wherein the barcoded identifier is directly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 27, the combination of Willis and Church makes obvious the method according to claim 25, and Willis further teaches wherein the barcoded identifier is indirectly attached to the substrate (para [0294], attachment or association with substrate).

Regarding claim 28, the combination of Willis and Church makes obvious the method according to claim 22, and Church further teaches the method further comprising, amplifying the barcoded identifier (para [0007]).

Regarding claim 29, the combination of Willis and Church makes obvious the method according to claim 22, and Church further teaches wherein sequencing is sequencing by synthesis (para [0045]).

Regarding claim 30, the combination of Willis and Church makes obvious the method according to claim 29, and Church further teaches wherein the sequencing by synthesis is single molecule sequencing by synthesis (para [0016]).

Regarding claim 31, the combination of Willis and Church makes obvious the method according to claim 22, and Willis further teaches wherein sequencing comprises:
hybridizing a primer to the barcoded identifier to form an identifier/primer duplex (para [0012], [0138]);
contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner (para [0228]-[0229]; [0138]);
detecting a signal from the incorporated labeled nucleotide (para [0225]-[0226]); and
sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the barcoded identifier nucleic acid (para [0225]-[0226]).

Regarding claim 32, the combination of Willis and Church makes obvious the method according to claim 31, and Willis further teaches wherein the detectably labeled nucleotide is an optically labeled nucleotide (para [0199])

Regarding claim 33, the combination of Willis and Church makes obvious the method according to claim 32, and Willis further teaches wherein the optically labeled nucleotide is a fluorescently labeled nucleotide (para [0199]).

Claims 1-33 have industrial applicability under PCT Article 33(4) because the subject matter can be made or used in industry.

SEARCH HISTORY

Application Number	PCT/US 12/29790
Search Conducted By	MAB
Search Approved By	RLS

US/IPC Classifications Searched	IPC(8): C12Q 1/68; C07H 21/04 (2012.01) USPC: 435/6.1, 536/23.1; 435/6.11, 436/94, 536/22.1, 536/24.3, 536/24.33
Date Conducted	21-25 May 2012 (21-25.05.2012)

Documentation Searched	IPC(8): C12Q 1/68; C07H 21/04 (2012.01) USPC: 435/6.1, 536/23.1; 435/6.11, 436/94, 536/22.1, 536/24.3, 536/24.33
Search Terms Used	Barcode, template, identifier, multiplex sequencing, identical, tag, validate, solution, nucleic acid, synthesis, substrate, contact, detect, polymerase, duplex, quantification, contamination, oligonucleotide
Date Conducted	21-25 May 2012 (21-25.05.2012)

Electronic Database Searched	PUBWEST
Files Searched	USPT, PGPB, EPAB, JPAB
Date Conducted	21-25 May 2012 (21-25.05.2012)

Search Logic:

DATE: Tuesday, May 22, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L13	L12 and (barcode$4 with (templat$4 and identif$4))	1	L13	L13
L12	L11 and barcode$2	1	L12	L12
L11	L10 and identif$4	33	L11	L11
L10	L9 and templat$4	34	L10	L10
L9	L8 and multiplex sequence$2	35	L9	L9
L8	L7 or L6	73187	L8	L8
L7	(435/6.1 or 536/23.1 or 435/6.11 or 436/94 or 536/22.1 or 536/24.3 or 536/24.33).ccls.	45882	L7	L7
L6	(C12Q1/68 or C07H21/04).ipc.	44032	L6	L6

END OF SEARCH HISTORY

DATE: Tuesday, May 22, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L5	L4 and (barcode$4 with (templat$4 and identif$4))	1	L5	L5
L4	L3 and barcode$4	1	L4	L4
L3	L2 and identif$4	44	L3	L3
L2	L1 and template$2	46	L2	L2
L1	multiplex sequence$4	171	L1	L1

END OF SEARCH HISTORY

DATE: Tuesday, May 22, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L21	L20 and (identifier with barcod$4)	2	L21	L21
L20	L19 and identifier	3	L20	L20
L19	L18 and (sequencing with multiplex)	21	L19	L19
L18	L17 and multiplex	34	L18	L18

L17	L14 and sequencing	55	L17	L17
L16	L15 and sequencing	55	L16	L16
L15	L14 and sequenc$4	160	L15	L15
L14	barcod$4 with (template$3 and identif$4)	228	L14	L14

END OF SEARCH HISTORY

DATE: Tuesday, May 22, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L22	identical with barcod$4 with templat$4 with identifier$2	0	L22	L22

END OF SEARCH HISTORY

DATE: Tuesday, May 22, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L5	L4 and ((tag$4 or barcod$4) with identical with (template$2 and identifier))	3	L5	L5
L4	L1 and ((barcod$4 or tag$2) with (identical$))	59	L4	L4
L3	L1 and ((barcod$4 or tag$2) with (identical))	59	L3	L3
L2	L1 and (validat$4 with templat$4)	0	L2	L2
L1	multiplex sequencing	335	L1	L1

END OF SEARCH HISTORY

DATE: Tuesday, May 22, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L10	L9 and barcode$2	7	L10	L10
L9	L7 and identifier	12	L9	L9
L8	L7 and (identifier with solution$2)	0	L8	L8
L7	L6 and (solution with template$2)	55	L7	L7
L6	multiplex sequenc$4	500	L6	L6

END OF SEARCH HISTORY

DATE: Wednesday, May 23, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L6	L5 and substrate$2	8	L6	L6
L5	L4 and (sequenc$4 with template$2 with identif$4)	8	L5	L5
L4	L3 and identifier	10	L4	L4
L3	L2 and (solution$2 with template$2)	37	L3	L3
L2	L1 and multiplex sequenc$4	246	L2	L2
L1	nucleic acid with template$2	24689	L1	L1

END OF SEARCH HISTORY

DATE: Wednesday, May 23, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L7	L6 and barcode$2	7	L7	L7
L6	L5 and substrate$2	8	L6	L6
L5	L4 and (sequenc$4 with template$2 with identif$4)	8	L5	L5
L4	L3 and identifier	10	L4	L4
L3	L2 and (solution$2 with template$2)	37	L3	L3
L2	L1 and multiplex sequenc$4	246	L2	L2
L1	nucleic acid with template$2	24689	L1	L1

END OF SEARCH HISTORY

DATE: Wednesday, May 23, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L8	L7 and synthesis	7	L8	L8
L7	L6 and barcode$2	7	L7	L7
L6	L5 and substrate$2	8	L6	L6
L5	L4 and (sequenc$4 with template$2 with identif$4)	8	L5	L5
L4	L3 and identifier	10	L4	L4
L3	L2 and (solution$2 with template$2)	37	L3	L3

L2	L1 and multiplex sequenc$4	246	L2	L2
L1	nucleic acid with template$2	24689	L1	L1

END OF SEARCH HISTORY

DATE: Wednesday, May 23, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L20	L19 and substrate$2	12	L20	L20
L19	L18 and solution$2	12	L19	L19
L18	L17 and (nucleic acid$2 with templat$4)	12	L18	L18
L17	L16 and (contact$4 with detect$4)	12	L17	L17
L16	L15 and polymerase$2	14	L16	L16
L15	L13 and duplex	14	L15	L15
L14	L13 and duplez	0	L14	L14
L13	L12 and (primer$2 with identifier$2)	22	L13	L13
L12	L11 and (primer$2 with template$2)	260	L12	L12
L11	multiplex sequencing	335	L11	L11
L10	L9 and (primer$2 with identifier$2)	0	L10	L10
L9	L8 and (primer$2 with template$2)	7	L9	L9
L8	L7 and synthesis	7	L8	L8
L7	L6 and barcode$2	7	L7	L7
L6	L5 and substrate$2	8	L6	L6
L5	L4 and (sequenc$4 with template$2 with identif$4)	8	L5	L5
L4	L3 and identifier	10	L4	L4
L3	L2 and (solution$2 with template$2)	37	L3	L3
L2	L1 and multiplex sequenc$4	246	L2	L2
L1	nucleic acid with template$2	24689	L1	L1

END OF SEARCH HISTORY

DATE: Wednesday, May 23, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L7	L6 and (barcode$2 with template$2 and (identifier$4 or tag$4))	13	L7	L7
L6	L5 and ((identifier$4 or tag$4) with templat$4)	50	L6	L6
L5	L4 and template$2	99	L5	L5
L4	L1 and contaminat$4	102	L4	L4

L3	L1 and contamination	71	L3	L3
L2	L1 and (quantif$4 with contamination)	0	L2	L2
L1	multiplex sequencing	335	L1	L1

END OF SEARCH HISTORY

DATE: Thursday, May 24, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L6	L2 and ((improper$4 or incorrect$4) with pair$4)	1	L6	L6
L5	L4 and ((improper$4 or incorrect$4) with pair$4)	0	L5	L5
L4	L3 and sequenc$4	8	L4	L4
L3	L2 and ((marker$2 or identifier$2) with barcode oligonucleotide$2)	8	L3	L3
L2	barcode$2 with oligonucleotide$2	199	L2	L2
L1	contamination with barcode$2 with oligonucleotide$2	0	L1	L1

END OF SEARCH HISTORY

DATE: Thursday, May 24, 2012 Purge Queries Printable Copy Create Case

Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ			
L12	improper$4 with pair$4 with barcode$2	0	L12	L12
L11	L9 and (improper$4 with pair$4)	0	L11	L11
L10	L9 and (improper$4 with pair$2)	0	L10	L10
L9	identif$4 with barcode with oligonucleotide$2	42	L9	L9
L8	identif$4 with barcode with oligonucleotide$2 with improper$4	0	L8	L8
L7	identif$4 with barcode with oligonucleotide$2 with improper$4 with pair$4	0	L7	L7

END OF SEARCH HISTORY

Electronic Database Searched	Google
Files Searched	Scholar
Date Conducted	21-25 May 2012 (21-25.05.2012)

Search Logic:

About 285 results (**0.10** sec) multiplex sequencing and identical barcode on template and identifier
About 129 results (**0.16** sec) validate integrity of nucleic acid template in multiplex sequencing and barcodes
About 1,520 results (**0.13** sec) validation of nucleic acid template and barcode
About 534 results (**0.10** sec) validation of nucleic acid template and barcode and multiplex sequencing
About 71 results (**0.11** sec) validate integrity of a NA template in a multiplex sequence reaction and barcode
About 1,590 results (**0.10** sec) validate integrity of a NA template in a multiplex sequence reaction
About 36 results (**0.08** sec) multiplex sequencing and validate integrity of a NA template in a multiplex sequence reaction and barcode
About 1,880 results (**0.09** sec) multiplex sequencing and template and identifier sequence
About 946 results (**0.13** sec) validate integrity of nucleic acid template and quantify contamination rate of multiplex sequencing
About 59 results (**0.13** sec) validate integrity of nucleic acid template and quantify contamination rate of multiplex sequencing and identical barcodes
About 492 results (**0.12** sec) quantify contamination rate of multiplex sequencing and identical barcodes
About 1,020 results (**0.10** sec) identify contamination in a batch of barcode oligonucleotides
About 143 results (**0.10** sec) identify improper pairing of barcodes and oligonucleotides
About 1,210 results (**0.13** sec) determine identify improper pairing of barcodes
About 412 results (**0.09** sec) incorrect pairing of barcode oligonucleotide
About 4,350 results (**0.37** sec) synthesis of oligonucleotide barcodes
About 1,630 results (**0.10** sec) synthesis of oligonucleotide barcodes contamination
About 2,130 results (**0.11** sec) barcode oligonucleotide contamination
About 3,850 results (**0.14** sec) barcode oligonucleotide and correct pairing
About 85 results (**0.10** sec) identify improperly paired barcode oligonucleotides
About 5,160 results (**0.14** sec)paired barcode oligonucleotide and sequencing

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

21710 7590 06/14/2013
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

NOTIFICATION DATE	DELIVERY MODE
06/14/2013	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 13/081,660	Applicant(s) PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 April 2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-46* is/are pending in the application.

5a) Of the above claim(s) *4,6,9,17-19,23,26,33,34,37,40 and 42-46* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-3,5,7,8,10-16,20-22,24,25,27-32,35,36,38,39 and 41* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *15 June 2011* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some * c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Interim copies:

a)☐ All b)☐ Some c)☐ None of the: Interim copies of the priority documents have been received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *5/24/12;8/6/12;5/6/13*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election of Group I (claims 1-41, species A, C and E) in the reply filed on April 30, 2013 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

2. Claims 4, 6, 9, 17-19, 23, 26, 33, 34, 37, 40 and 42-46 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species and invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on April 30, 2013.

3. Claims 1-3, 5, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, 39 and 41 will be examined.

Information Disclosure Statement

4. The information disclosure statements (IDSs) submitted on May 24, 2012 and August 6, 2012 are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

5. The information disclosure statement (IDS) submitted on May 6, 2013 was filed after the mailing date of the Restriction/Election Requirement on April 18, 2013. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Interpretation

6. Applicant did not define the terms "sequence identifiers" or "barcode sequences" therefore they are interpreted as any known sequences.

Claim Rejections - 35 USC § 112

7. The following is a quotation of 35 U.S.C. 112(b):

(B) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 31 and 32 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 31 recites the limitation "the detectably labeled nucleotide" in line 1. There is insufficient antecedent basis for this limitation in the claim. Claims 20, 28 and 29, from which claim 31 depends, do not contain a limitation “a detectably labeled nucleotide”.

Claim Rejections - 35 USC § 102

9. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

10. Claims 1-3, 5, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, 39 and 41 are rejected under 35 U.S.C. 102(b) as being anticipated by Kawashima et al. (US 2005/0100900 A1; published May 2005).

Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Kawashima et al. teach a method comprising:

obtaining a nucleic acid template (page 4, [0049]-[0051]; [0056]);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (page 2, [0026]-[0029]; page 4, [0052]-[0053]; Fig. 15, page 13, [0182]-[0189], where the tag sequences are the barcodes);

amplifying the template (Fig. 1; page 6, [0109]; page 7, [0110]-[0124]; page 10, [0149]-[0157]); and

sequencing the template (Fig. 17; page 15-18).

Regarding claims 3, 22 and 36, Kawashima et al. teach the template having the following configuration: 5'-P1 primer (=first known sequence)- tag (=a first barcode sequence)-S1 (=a second known sequence)-target sequence-S2 (=a third known sequence)-tag (= a second barcode sequence)-P2 primer (= a fourth known sequence) (Fig. 15d, Fig. 16b).

Regarding claims 5 and 39, Kawashima et al. teach the tag sequences being the same (Fig. 15d, Fig. 16b; Page 13, [00183], where it is specified that the tag identifies the sample, but not the nucleic acid strand).

Regarding claims 7, 8, 24, 25 and 38, Kawashima et al. teach the template directly immobilized on a solid support (Fig. 1).

Regarding claims 11, 12, 28 and 29, Kawashima et al. teach single molecule sequencing by synthesis (Fig. 17, page 5, [0067]-[0075]; page 15, [0233]-[0238]).

Regarding claims 13, 30 and 41, Kawashima et al. teach hybridizing a primer to the template, contacting the duplex with a polymerase in the presence of detectably labeled

nucleotide, detecting the signal from incorporated nucleotide and repeating the process to determine the sequence of nucleic acid (Fig. 17; page 15, [0238]-[0245]).

Regarding claims 14, 15, 31 and 32, Kawashima et al. teach fluorescently labeled nucleotides (page 15, [0239]-[0241]).

11. No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TERESA E STRZELECKA
Primary Examiner
Art Unit 1637

/TERESA E STRZELECKA/
Primary Examiner, Art Unit 1637
June 11, 2013

Notice of References Cited	Application/Control No. 13/081,660	Applicant(s)/Patent Under Reexamination PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2005/0100900	05-2005	Kawashima et al.	435/006
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

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	N					
	O					
	P					
	Q					
	R					
	S					
	T					

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*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013								
	1	✓								
	2	✓								
	3	✓								
	4	N								
	5	✓								
	6	N								
	7	✓								
	8	✓								
	9	N								
	10	✓								
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	30	✓								
	31	✓								
	32	✓								
	33	N								
	34	N								
	35	✓								
	36	✓								

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013								
	37	N								
	38	✓								
	39	✓								
	40	N								
	41	✓								
	42	N								
	43	N								
	44	N								
	45	N								
	46	N								

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Porreca	Gregory	Search

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	13/081,660 - Conf. No. 4441
	Filing Date	04/07/2011
	First Named Inventor	Gregory PORRECA
	Art Unit	1634
	Examiner Name	Teresa Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
/TS/	1	6,403,320	B1	06-2002	Read et al.	
If you wish to add additional U.S. Patent citation information please click the Add button.						

U.S.PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
/TS/	1	2009/0163366	A1	06-2009	Nickerson et al.	
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FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
/TS/	1	WO 2009/036525	WO	A2	03/26/2009	CUPPENS, Harry		☐
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NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

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WEST Search History for Application 13081660

Creation Date: 2013061015:10

Prior Art Searches

Query	DB	Op.	Plur.	Thes.	Date
6403320.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
20090163366	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20080269068	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20100035243 or 20100297626 or 20100330619	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20070166705	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20090226975	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20050100900	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
7115400.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
7057026.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parrallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6 or barcod$5) and sequencing	USPT	ADJ	YES	ASSIGNEE	06-10-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6 or barcod$5) and sequencing	USPT	ADJ	YES	ASSIGNEE	06-10-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6 or barcod$5) and sequencing	PGPB	ADJ	YES	ASSIGNEE	06-10-2013

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CONFIRMATION NO. 4441

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/081,660	04/07/2011 RULE	435	1637	GSGE-001/01US 29258/14

APPLICANTS

Gregory Porreca, Cambridge, MA;
Mark Umbarger, Brookline, MA;
George Church, Brookline, MA;

**** CONTINUING DATA **************************

This appln claims benefit of 61/426,817 12/23/2010

**** FOREIGN APPLICATIONS **************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **

04/14/2011

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged /TERESA E STRZELECKA/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		MA	3	46	4

ADDRESS

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111
UNITED STATES

TITLE

METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

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1313	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

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	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

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/TS/	1	20100330619		2010-12-30	Willis et al.	
	2	20080269068		2008-10-30	CHURCH et al.	duplicate

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	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
/TS/	1	International Search Report and Written Opinion for PCT/US12/29790, mailed June 14, 2012 17 pages	☐

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Examiner Signature	/Teresa Strzelecka/	Date Considered	06/07/2013

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[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

PTO/SB/08a (01-10)
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	Art Unit	1637
	Examiner Name	STRZELECKA, Teresa
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS

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U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
/TS/	1	20080269068		2008-10-30	Church	
/TS/	2	20100035243		2010-02-11	Muller	
/TS/	3	20100297626		2010-11-25	McKernan	

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FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	PORRECA, Gregory
	Art Unit	1637
	Examiner Name	STRZELECKA, Teresa
	Attorney Docket Number	GSGE-001/01US 29258/14

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
/TS/	1	International Search Report and Written Opinion in corresponding international application PCT/US2011/065098, filed 15 December 2011, mailed 03 April 2012 (10 pages)	☐

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EXAMINER SIGNATURE

Examiner Signature	/Teresa Strzelecka/	Date Considered	06/07/2013

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
IDS (5/24/12; 8/6/12; 5/6/13)	6/7/13	/TS/
Inventor name search	6/7/13	/TS/
WEST	6/7/13, 6/10/13	/TS/

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Porreca et al.	ART UNIT:	1637
SERIAL NUMBER:	13/081,660	CONFIRMATION NO.:	4441
FILING DATE:	April 7, 2011	EXAMINER:	Strzelecka, Teresa E.
TITLE:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION		

FILED ELECTRONICALLY

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE

This paper is in response to a non-final Office Action mailed June 14, 2013 from the United States Patent and Trademark Office.

Applicants respectfully petition for and request a three-month extension of time to bring the response deadline up to and including Monday, December 16, 2013. Applicants authorize the Commissioner to charge the extension fee and any other fees due to Deposit Account No. 500369.

Amendments to the specification begin on page 2.

Amendments to the claims begin on page 3.

Remarks begin on page 10.

Amendments to the specification

No amendments to the specification are made herein.

Amendment to the claims

Please amend claims 1, 20, 21, 31, and 35 as follows. This list of claims replaces all prior versions and listings of claims in this application.

1. (Currently amended) A method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction, the method comprising:

obtaining a template nucleic acid;

incorporating at least two sequence identifiers into the template; ~~and~~

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the two incorporated sequence identifiers remain associated with the template, thereby validating the integrity of the nucleic acid template in a multiplex sequencing reaction.

2. (Original) The method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

3. (Original) The method according to claim 2, wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

4. (Withdrawn) The method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

5. (Original) The method according to claim 2, wherein the first and second identifiers are the same.

6. (Withdrawn) The method according to claim 2, wherein the first and second identifiers are different.

7. (Original) The method according to claim 1, wherein prior to sequencing, the template is attached to a substrate.

8. (Original) The method according to claim 2, wherein the template is directly attached to the substrate.

9. (Withdrawn) The method according to claim 2, wherein the template is indirectly attached to the substrate.

10. (Original) The method according to claim 7, further comprising, amplifying the template.

11. (Original) The method according to claim 1, wherein sequencing is sequencing by synthesis.

12. (Original) The method according to claim 11, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

13. (Original) The method according to claim 1, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

14. (Original) The method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

15. (Original) The method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

16. (Original) The method according to claim 1, wherein the pair of identifiers are first and second barcode sequences that are attached to the template.

17. (Withdrawn) The method according to claim 16, wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template.

18. (Original) The method according to claim 17, wherein the first and second barcode sequences are the same.

19. (Withdrawn) The method according to claim 17, wherein the first and second barcode sequences are different.

20. (Currently amended) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a pair of barcode sequences to the template; ~~and~~

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

21. (Currently amended) The method according to claim 20, wherein a first ~~identifier~~ barcode sequence is incorporated into a 5' portion of the template and a second ~~identifier~~ barcode sequence is incorporated into a 3' portion of the template.

22. (Original) The method according to claim 21, wherein after attaching of the pair of barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

23. (Withdrawn) The method according to claim 20, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

24. (Original) The method according to claim 20, wherein prior to sequencing, the template is attached to a substrate.

25. (Original) The method according to claim 24, wherein the template is directly attached to the substrate.

26. (Withdrawn) The method according to claim 24, wherein the template is indirectly attached to the substrate.

27. (Original) The method according to claim 24, further comprising, amplifying the template.

28. (Original) The method according to claim 20, wherein sequencing is sequencing by synthesis.

29. (Original) The method according to claim 28, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

30. (Original) The method according to claim 20, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

31. (Currently amended) The method according to claim ~~29~~ 30, wherein the ~~detectably~~ labeled nucleotide is an optically labeled nucleotide.

32. (Original) The method according to claim 31, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

33. (Original) The method according to claim 23, wherein the first and second barcode sequences are the same.

34. (Withdrawn) The method according to claim 23, wherein the first and second barcode sequences are different.

35. (Currently amended) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template;

amplifying the template; ~~and~~

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

36. (Original) The method according to claim 35, wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

37. (Withdrawn) The method according to claim 35, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

38. (Original) The method according to claim 35, wherein prior to amplifying, the template is immobilized to a solid support.

39. (Original) The method according to claim 35, wherein the first and second barcode sequences are the same.

40. (Withdrawn) The method according to claim 35, wherein the first and second barcode sequences are different.

41. (Original) The method according to claim 35, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

42. (Withdrawn) A method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte.

43. (Withdrawn) The method of claim 42, wherein said identifiers are nucleic acid sequence tags.

44. (Withdrawn) The method of claim 43, wherein said sequence tags are associated with a moiety that binds said analyte.

45. (Withdrawn) The method of claim 42, wherein said analyte is a protein.

46. (Withdrawn) The method of claim 45, wherein said identifiers are peptides.

Remarks

Claims 1, 20, and 35 are amended to recite the step of analyzing sequence reads to determine if two incorporated sequence identifiers remain associated with a template. Support for that amendment is found at pp. 6-8 and Figure 2 of the as-filed application. Claims 21 and 31 are amended to correct a clerical errors in each of those claims. No new matter is added.

Clarity of Claims

The Office Action rejects claims 31-32 under 35 U.S.C. §112 ¶2 stating that the phrase "the detectably labeled nucleotide" in claim 31 lacks antecedent basis. Claim 31 is amended to depend from claim 30 and now recites "the labeled nucleotide", which phrase has proper antecedent basis in claim 30. Applicants respectfully request withdrawal of that rejection.

Novelty of Claims

The Office Action rejects claims 1-3, 5, 7-8, 10-16, 20-22, 24-25, 27-32, 35-36, 38-39, and 41 under 35 U.S.C. §102(b) in view of Kawashima (U.S. patent application number 2005/01000900). Applicants respectfully disagree.

The as-filed application on pp. 1-2 explains that a problem in sequencing reactions, particularly multiplex sequencing reactions, is maintaining accurate identification of a nucleic acid template from a sample throughout the sequencing process. That section of the as-filed application explains that errors can occur during generation of the library, arraying and amplification steps that result in improper pairing of template strands from different samples. Molecular overlap during sequencing sample preparation may lead to assigning sequencing data to the wrong patient sample. The as-filed application at pp. 2 and 6-7 and Figure 2 explains that the solution to that problem utilizes two or more identifiers that are uniquely associated with a nucleic acid template of interest, such that the identifiers will only be present when the analyte of interest is detected. In that way, false positive and/or false negative results are avoided by requiring concomitant detection of two or more independent markers associated with the nucleic

acid template to be detected. To better reflect that solution, the independent claims are amended to recite the element of analyzing sequence reads to determine if two incorporated sequence identifiers remain associated with a template. Claims now require concomitant detection of two or more independent markers associated with the nucleic acid template to be detected.

In contrast to the amended claims, Kawashima is limited to reporting different sequencing methods. Kawashima only reports annealing templates to primers and optionally then re-sequencing those templates with a second primer (Kawashima, paragraphs [0016] to [0108] and FIGs. 1A, 1B, 3A, 6A-6B, 9A-9B, 10A-10B, 12A-12C, 13A-13B, 14A-14B, and 16A-16B). Kawashima cannot possibly be an anticipatory reference against the amended claims, as Kawashima's methods do not analyze sequence reads to determine if identifiers remain associated with a template after a sequencing reaction for the purpose of avoiding false positive and/or false negative results. See M.P.E.P. §2131 which states that a reference must show each and every element of a claim to be used as a reference against the novelty of that claim.

In fact, Kawashima does not contemplate that molecular overlap or mismatching can occur during a sequencing reaction. Therefore Kawashima neither contemplates the problem addressed by the pending claims nor provides any solution to that problem, particularly the solution provided by the amended claims. Rather, Kawashima assumes that all results of his sequencing reactions are accurate.

For at least those amendments and reasons, the amended claims are novel in view of Kawashima. Applicants respectfully request withdrawal of the 35 U.S.C. §102(b) rejection.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: December 13, 2013

Respectfully submitted,
BROWN RUDNICK LLP

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

61350549

Electronic Patent Application Fee Transmittal

Application Number:	13081660
Filing Date:	07-Apr-2011
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Filer:	Thomas C. Meyers/Diane Riordan
Attorney Docket Number:	GSGE-001/01US 29258/14

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	2253	1	700	700

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
		Total in USD ($)		**700**

Electronic Acknowledgement Receipt	
EFS ID:	17650873
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Diane Riordan
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	13-DEC-2013
Filing Date:	07-APR-2011
Time Stamp:	10:49:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$700
RAM confirmation Number	10678
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		GSGE_001_01US_Amendment_and_response_to_OA_mailed_6_14_2013.pdf	53190 a2aac76d17a543e87513cfa4c866e3bbe8fbc1a0	yes	12

	Multipart Description/PDF files in .zip description		
	Document Description	Start	End
	Amendment/Req. Reconsideration-After Non-Final Reject	1	1
	Specification	2	2
	Claims	3	9
	Applicant Arguments/Remarks Made in an Amendment	10	12

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30624 7b8c6f110e535ab96ff13eaa9add3372612dbd4c	no	2

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/081,660	Filing Date 04/07/2011	☒ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT 12/13/2013	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 46	Minus	** 46	= 0	X $40 =	0
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $210 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
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The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/ANGELA JOHNSON/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

21710 7590 03/07/2014
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

NOTIFICATION DATE	DELIVERY MODE
03/07/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

Office Action Summary	Application No. 13/081,660	Applicant(s) PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12/13/13*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-46* is/are pending in the application.

5a) Of the above claim(s) *4,6,9,17-19,23,26,34,37,40 and 42-46* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-3,5,7,8,10-16,20-22,24,25,27-33,35,36,38,39 and 41* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

1. The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

2. This office action is in response to an amendment filed December 13, 2013. Claims 1-46 were previously pending, with claims 4, 6, 9, 17-19, 23, 26, 33, 34, 37, 40 and 42-46 withdrawn from consideration. Applicant amended claims 1, 20, 31 and 35. Claims 1-3, 5, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, 39 and 41 will be examined.

3. Applicant's amendments overcame the previously presented rejections. This office action contains new grounds for rejection necessitated by amendment.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 1-3, 7, 8, 10-12, 16, 20-22, 24, 25, 27-29, 35, 36 and 38 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Parameswaran et al. (Nucl. Acids Res., vol. 35, e130, pp. 1-9, 2007).

Claims 1, 2, 10, 16, 20, 21, 27 and 35 are considered together in claim 35, since it contains limitations of all of these claims.

Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Parameswaran et al. teach a method comprising:

obtaining a nucleic acid template (page 2, third paragraph);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (page 2, paragraphs 4-6; page 3; page 4, first and second paragraphs; Fig. 1);

amplifying the template (page 4, third paragraph);

sequencing the template to generate sequence reads (page 4, third paragraph), and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with a template (page 7, paragraphs 2-4; page 8, first and second paragraph; Table 3).

Regarding claims 3, 22 and 36, Parameswaran et al. teach the template having the following configuration: 5'-F-adapter (=first known sequence)- F-barcode (=a first barcode sequence)-F-cloning linker (=a second known sequence)-target sequence-RC (R-cloning linker) (=a third known sequence)-RC (R-barcode) (= a second barcode sequence)-RC(R-adapter) (= a fourth known sequence) (Fig. 1).

Regarding claims 7, 8, 24, 25 and 38, Parameswaran et al. teach the template undergoing emulsion PCR, therefore they inherently teach template directly immobilized on a solid support (page 4, third paragraph).

Regarding claims 11, 12, 28 and 29, Parameswaran et al. teach pyrosequencing (page 4, third paragraph), therefore they inherently teach single molecule sequencing by synthesis, since each DNA molecule is sequenced individually.

Claim Rejections - 35 USC § 103

6. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 1-3, 5, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, 39 and 41 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kawashima et al. (US 2005/0100900 A1; published May 2005; previously cited), Parameswaran et al. (Nucl. Acids Res., vol. 35, e130, pp. 1-9, 2007) and Kircher et al. (Bioessays, vol. 32, pp. 524-536, June 2010).

A) Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Kawashima et al. teach a method comprising:

obtaining a nucleic acid template (page 4, [0049]-[0051]; [0056]);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (page 2, [0026]-[0029]; page 4, [0052]-[0053]; Fig. 15, page 13, [0182]-[0189], where the tag sequences are the barcodes);

amplifying the template (Fig. 1; page 6, [0109]; page 7, [0110]-[0124]; page 10, [0149]-[0157]); and

sequencing the template (Fig. 17; page 15-18).

Regarding claims 3, 22 and 36, Kawashima et al. teach the template having the following configuration: 5'-P1 primer (=first known sequence)- tag (=a first barcode sequence)-S1 (=a second known sequence)-target sequence-S2 (=a third known sequence)-tag (= a second barcode sequence)-P2 primer (= a fourth known sequence) (Fig. 15d, Fig. 16b).

Regarding claims 5 and 39, Kawashima et al. teach the tag sequences being the same (Fig. 15d, Fig. 16b; Page 13, [00183], where it is specified that the tag identifies the sample, but not the nucleic acid strand).

Regarding claims 7, 8, 24, 25 and 38, Kawashima et al. teach the template directly immobilized on a solid support (Fig. 1).

Regarding claims 11, 12, 28 and 29, Kawashima et al. teach single molecule sequencing by synthesis (Fig. 17, page 5, [0067]-[0075]; page 15, [0233]-[0238]).

Regarding claims 13, 30 and 41, Kawashima et al. teach hybridizing a primer to the template, contacting the duplex with a polymerase in the presence of detectably labeled nucleotide, detecting the signal from incorporated nucleotide and repeating the process to determine the sequence of nucleic acid (Fig. 17; page 15, [0238]-[0245]).

Regarding claims 14, 15, 31 and 32, Kawashima et al. teach fluorescently labeled nucleotides (page 15, [0239]-[0241]).

B) Kawashima et al. do not teach that the barcodes can be used to validate the integrity of nucleic acid templates.

C) Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Parameswaran et al. teach a method comprising:

obtaining a nucleic acid template (page 2, third paragraph);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (page 2, paragraphs 4-6; page 3; page 4, first and second paragraphs; Fig. 1);

amplifying the template (page 4, third paragraph);

sequencing the template to generate sequence reads (page 4, third paragraph), and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with a template (page 7, paragraphs 2-4; page 8, first and second paragraph; Table 3).

Regarding claims 3, 22 and 36, Parameswaran et al. teach the template having the following configuration: 5'-F-adapter (=first known sequence)- F-barcode (=a first barcode sequence)-F-cloning linker (=a second known sequence)-target sequence-RC (R-cloning linker) (=a third known sequence)-RC (R-barcode) (= a second barcode sequence)-RC(R-adapter) (= a fourth known sequence) (Fig. 1).

Regarding claims 7, 8, 24, 25 and 38, Parameswaran et al. teach the template undergoing emulsion PCR, therefore they inherently teach template directly immobilized on a solid support (page 4, third paragraph).

Regarding claims 11, 12, 28 and 29, Parameswaran et al. teach pyrosequencing (page 4, third paragraph), therefore they inherently teach single molecule sequencing by synthesis, since each DNA molecule is sequenced individually.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the barcodes of Kawashima et al. to validate the integrity of the nucleic acid molecules being sequenced, as suggested by Parameswaran et al. (page 8, last paragraph):

"Our barcode specification has been tailored for use with pyrosequencing technologies, particularly reflecting restrictions imposed on the generation of homopolymeric runs. These barcodes may also be compatible with other sequencing platforms. Optimally, however the precise structure of the barcodes might need to be revised to reflect sequencing error profiles of

the various systems. If necessary, multiple sequencing primers may also be used to identify the barcode and the sequence of interest, to overcome limitations imposed by short read lengths. As the availability of high-throughput technology advances, massively multiplexed barcoded sequencing will be extremely useful in performing surveys of diverse systems for nucleic acid signatures."

As stated by Kircher et al. regarding the type of sequencing performed by the method of Kawashima et al. (page 527, last three paragraphs; page 528; Fig. 3):

"The Illumina library and flow cell preparation includes several in vitro amplification steps, which cause a high background error rate and contribute to the average error rate of about 10^{-2}–10^{-3} [41, 42]. Further, the flow cell preparation creates a fraction of ordinary-looking clusters that are initiated from more than one individual sequence. These results in mixed signals and mostly low quality sequences for these clusters. Similar to the 454 ghost wells, the Illumina image analysis may identify chemistry crystals, dust, and lint particles as clusters and call sequences from these. In such cases the resulting sequences typically appear to be of low sequence complexity."

Therefore one of ordinary skill in the art at the time of the invention would have been motivated to use error detection using barcode sequences to verify correctness of sequencing results as suggested by Parameswaran et al. in the method of Kawashima et al. to avoid sequencing errors resulting from sample preparation and processing described by Kircher et al.

8. No claims are allowed.

Conclusion

9. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TERESA E STRZELECKA
Primary Examiner
Art Unit 1637

/TERESA E STRZELECKA/
Primary Examiner, Art Unit 1637
February 28, 2014

Notice of References Cited	Application/Control No. 13/081,660	Applicant(s)/Patent Under Reexamination PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Parameswaran, P. et al., Nucl. Acids Res., vol. 35, e130, pp. 1-9 (2007)
	V	Kircher, M. et al., Bioessays, vol. 32, pp. 524-536 (June 2010)
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013	02/28/2014							
	1	✓	✓							
	2	✓	✓							
	3	✓	✓							
	4	N	N							
	5	✓	✓							
	6	N	N							
	7	✓	✓							
	8	✓	✓							
	9	N	N							
	10	✓	✓							
	11	✓	✓							
	12	✓	✓							
	13	✓	✓							
	14	✓	✓							
	15	✓	✓							
	16	✓	✓							
	17	N	N							
	18	N	N							
	19	N	N							
	20	✓	✓							
	21	✓	✓							
	22	✓	✓							
	23	N	N							
	24	✓	✓							
	25	✓	✓							
	26	N	N							
	27	✓	✓							
	28	✓	✓							
	29	✓	✓							
	30	✓	✓							
	31	✓	✓							
	32	✓	✓							
	33	N	N							
	34	N	N							
	35	✓	✓							
	36	✓	✓							

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	**Art Unit**
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013	02/28/2014							
	37	N	N							
	38	✓	✓							
	39	✓	✓							
	40	N	N							
	41	✓	✓							
	42	N	N							
	43	N	N							
	44	N	N							
	45	N	N							
	46	N	N							

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference G0814.70000	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2010/001293	International filing date *(day/month/year)* 30 April 2010 (30.04.2010)	Priority date *(day/month/year)* 30 April 2009 (30.04.2009)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GOOD START GENETICS, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 01 November 2011 (01.11.2011)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Philippe Bécamel e-mail: pt01.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK R.H. WALLER
WOLF, GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	28 OCT 2010
Applicant's or agent's file reference G0814.70000	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 10/01293	30 April 2010 (30.04.2010)	30 April 2009 (30.04.2009)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/94

Applicant GOOD START GENETICS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	07 Oct. 2010 (07.10.2010)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 15, 16, 19, 22-56, 64, 65, 67

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 15, 16, 19, 22-56, 64, 65, 67 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims are not formed according to the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 15, 16, 19, 22-56, 64, 65, 67

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☒ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-8, and 57, directed to a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different.

Group II: claims 9-14, 17, 18, and 66, directed to a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject.

Group III: claims 20-21, directed to a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject.

Group IV: claims 58-63, directed to a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The inventions listed as Groups I - IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different. The special technical feature of the Group II claims is a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject. The special technical feature of the Group III claims is a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject. The special technical feature of the Group IV claims is a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract; para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface). Therefore, the inventions of Groups I - IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-14, 17, 18, 57 and 66

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-14, 17, 18, 57 and 66	YES
	Claims	None	NO
Inventive step (IS)	Claims	17, 18, 57	YES
	Claims	1-14 and 66	NO
Industrial applicability (IA)	Claims	1-14, 17, 18, 57 and 66	YES
	Claims	None	NO

2. Citations and explanations:

Claim 66 lacks an inventive step under PCT Article 33(3) as being obvious over the article entitled 'Multiplexed genotyping with sequence-tagged molecular inversion probes' by Hardenbol et al. (hereinafter 'Hardenbol').

As per claim 66, Hardenbol discloses a method of genotyping a nucleic acid in a biological sample, the method comprising: obtaining a nucleic acid preparation using a preparative method on a biological sample (pg 674, col 2, para 2--25 different individuals were genotyped, pg 677, col 1, para 3), sequencing a target nucleic acid of the nucleic acid preparation (pg 674, col 2, para 3), and performing a molecular inversion probe capture reaction on the biological sample (pg 677, col 2, para 2), genotyping the target nucleic acid based on the results of the capture reaction (pg 674, col 2, para 2). Hardenbol further teaches that the MIPs are engineered such that when annealed to a target nucleic acid, a gap between the flanking regions comprises only one nucleotide for detection of a single nucleotide polymorphism (pg 674, col 1, para 2) and it would have been obvious to one skilled in the art during the polymerization reaction to add only the nucleotide that would have indicated an SNP at this locus if the molecular inversion probe captured the target nucleic acid in the biological sample to save reagents and time. It further would have been obvious to one skilled in the art to utilize the sequence information in addition to the capture reaction information to ensure accurate genotyping.

Claim 9 lacks an inventive step under PCT Article 33(3) as being obvious over US 2003/0203370 A1 to Yakhini et al. (hereinafter "Yakhini").

As per claim 9, although Yakhini does not explicitly teach a method of genotyping a subject, the method comprising: determining the sequence of at least a threshold number of independently isolated nucleic acids, wherein the sequence of each isolated nucleic acid comprises a target nucleic acid sequence and a differentiator tag sequence, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences, wherein the isolated nucleic acids are identified as independently isolated if they comprise unique combinations of target nucleic acid and differentiator tag sequences, and wherein the target nucleic acid sequence is the sequence of a genomic locus of a subject, Yakhini discloses a method of genotyping using mass tags (abstract), wherein "As discussed above, there are generally a much smaller number of mass tags than primer sequences, and many different primers may be therefore identically labeled. Thus, fingerprinting SNPs using the CMT/PEA technique involves extracting a small fingerprint signal from complex mass spectra in which the presence of a particular mass tag may indicate extension of many different oligonucleotide primers. Moreover, even considering only SNP-characteristic primers, there may be a number of different genotypes that map to a particular mass spectrum. For example, the mass spectrum shown in FIG. 10 corresponds to mass tags removed from primers, extended as shown in FIG. 8, characteristic for the genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6. However, the same mass spectrum is produced by the above-illustrated CMT/PEA technique when applied to the example genotype S1/S1', S2/S2', S3/S3', S4'/S4', S5/S5', S6/S6, as can be see noting that, in FIG. 6, the characteristic primers for polymorphism S3 610-613 carry the same mass tags as the primer sequences characteristic for polymorphism S4' 614-617 and the characteristic primers for polymorphism S3' carry the same mass tags as the primer sequences characteristic for polymorphism S4. Therefore, the genotyping assay illustrated in FIGS. 4-10 needs to be carried out in multiple assay steps in order to distinguish genotype S1/S1', S2/S2', S3/S3, S4/S4, S5/S5', S6/S6 from genotype S1/S1', S2/S2', S3'/S3', S4'/S4', S5/S5', S6/S6. Instead of hybridizing the set of oligonucleotide primers to all chromosome fragments, including fragments produced from all six polymorphisms, the chromosome fragments need to be partitioned into sets that generate unambiguous mask-tag fingerprints under the CMT/PEA assay technique". (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini.

-----Continued in Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V(2): Citations and Explanations

Claims 1- 5, and 7 lack an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of the article entitled 'Applications of DNA tiling arrays for whole-genome analysis' by Mockler et al. (hereinafter 'Mockler').

As per claim 1, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising:
contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3-1,517 loci were genotyped in a 1,517-probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid (Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein each subregion overlaps with at least one other subregion. Mockler, however, discloses wherein each subregion overlaps with at least one other subregion (Fig. 2a). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 2, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler further teaches wherein at least one subregion entirely overlaps at least one other subregion (Fig. 2D-probes tiled at 1 base resolution (if these were MIPs, as taught by Hardenbol, at least two of the nucleotides would be flanking nucleotides and thus one subregion would entirely overlap one other subregion)).

As per claim 3, the combination of Hardenbol and Mockler disclose the method of claim 1, and Mockler discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, non-overlapping with the sequences of the 5' region and the 3' region of each other probe (Fig. 2B).

As per claim 4, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol discloses wherein the sequences of the 5' region and the 3' region of each probe are, respectively, different than the sequences of the 5' region and the 3' region of each other probe (Fig. 2a-H1 and H2 unique to each probe).

As per claim 5, Hardenbol discloses a method of analyzing a plurality of genetic loci, the method comprising: contacting each of a plurality of target nucleic acids with a probe set (abstract, pg 673, col 2, para 2, Fig. 2; pg 674, col 2, para 3--1,517 loci were genotyped in a 1,517 probe multiplex analysis), wherein each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid Fig. 2a, b; pg 674, col 2, para 3), wherein the subregions of the target nucleic acid are different (pg 674, col 2, para 2-3--probes were generated for 1,121 SNPs. 1,517 loci were genotyped in a 1,517-probe multiplex analysis), isolating a plurality of nucleic acids each having a nucleic acid sequence of a different subregion for each of the plurality of target nucleic acids, and analyzing the isolated nucleic acids (pg 674, col 1, para 2 to pg 674, col 2, para 1), but fails to specifically disclose wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe. Mockler, however, discloses a tiling array wherein a portion of the 5' region and a portion of the 3' region of a probe have, respectively, the sequence of the 5' region and the 3' region of a different probe (Fig. 2a, d). It would have been obvious to one skilled in the art to use the tiling approach of Mockler in the method of Hardenbol, as Mockler teaches wherein such an approach reduces bias (pg 2, col 1, para 4 to pg 2, col 2, para 1).

As per claim 7, the combination of Hardenbol and Mockler disclose the method of claim 1, and Hardenbol further teaches wherein the analyzing comprises genotyping a polymorphism of the target nucleic acid (abstract).

Claims 10-14 lack an inventive step under PCT Article 33(3) as being obvious over Yakhihi in view of Hardenbol.

As to claim 10, although Yakhini does not explicitly teach wherein the isolated nucleic acids are molecular inversion probe capture products, Hardenbol teaches the use of said products for the genotyping of SNP's (abstract). It would have been obvious to a person skilled in the art that the mollecular inversion probe capture products taught by Hardenbol could have substituted for the mass spectrometric tags taught by Yakhini, with a similar type of degeneracy requiring the determination of sets to generate unambiguous sequence-tag fingerprints (para [0026]) in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

------Continued in Subsequent Supplemental Box-------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 10/01293

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V.2: Citations and Explanations

As to claim 11, Hardenbol discloses a method of genotyping a subject, the method comprising:
determining the sequence of molecular inversion probe capture products (abstract, analyzed using universal sequence tag DNA microarrays), each comprising a molecular inversion probe and a target nucleic acid (Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a--P1,P2,Tag), and wherein the target nucleic acid is a captured genomic locus of a subject (pg 674, col 2, para 2), and genotyping the subject at the captured genomic locus based on the sequence of the captured target nucleic acid (abstract, pg 674, col 2, para 2), but fails to specifically teach genotyping the subject at the captured genomic locus based on the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequences of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 12, Hardenbol discloses a method of genotyping a subject, the method comprising: obtaining molecular inversion probe capture products, each comprising a molecular inversion probe and a target nucleic acid , wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), wherein the target nucleic acid is a captured genomic locus of the subject (abstract, pg 674, col 2, para 2), amplifying the molecular inversion probe capture products (pg 674, col 1, para 2), and genotyping the subject by determining, for each target nucleic acid, the sequence of the target nucleic acid (abstract--analzed using unversal sequence tag DNA microarrays), but fails to specifically disclose genotyping the subject by determining, for each target nucleic acid, the sequence of at least a threshold number of unique combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products. However, Yakhini teaches a method of genotyping a subject using target nucleic acids linked to differentiator tags (abstract), wherein unique set of tags are identified to generate unambiguous sequence-tag fingerprints (para [0026]). Although Yakhihi does not explicitly teach the determination of a minimum or threshold number associated with the partitioning, a person skilled in the art would have desired to do so in order to enable automation of the process, in particular computerization of the partitioning into sets that generate unambiguous fingerprints, as taught by Yakhini. It would have been obvious to a person skilled in the art to combine the teaching of Hardenbol with that of Yakhini in order to scale up the procedure taught by Yakhini to a large number of target nucleic acids.

As to claim 13, Hardenbol discloses wherein the obtaining comprises capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, each comprising a unique differentiator tag sequence (Each amplified probe contains a unique tag sequence that is complementary to a sequence on the universal tag array; pg. 270, col 1, para 2).

As to claim 14, although neither Hardenbol nor Yakhini explcitly teaches wherein the capturing is performed under conditions wherein the likelihood of obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences is equal to or less than a predetermined value, optionally wherein the predetermined value is about 0.05, Hardenbol teaches wherein repeatability and trio accuracy were greater than 99%, which corresponds to an error rate of less than 0.01. A person skilled in the art would have known that a likelihood of ambiguity (obtaining two or more molecular inversion probe capture products with identical combinations of target and differentiator tag sequences) of the results for a genotyping assay, particularly assays associated with either prognostication of disease or of the potential utility of pharmaceuticals to treat a disease, would have been required to be at least less than 5%.

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over Hardenbol in view of Mockler, further in view of US 2008/0280955 A1 to McCamish et al. (hereinafter 'McCamish').

As per claim 6, the combination of Hardenbol and Mockler discloses the method of claim 1, but fails to teach that which McCamish teaches, that is wherein the target nucleic acids are selected from Table 1 (Table 9- NC_000011.8 (DHCR7), NC_000016.8 (GCSH), NC_000019.8 (BCKDH), NC_000021.7 (HLCS)). As McCamish teaches that the SNPs in Table 9 are related to drug response to blood glucose regulation (para [0258], claims 1, 15), it would have been obvious to one skilled in the art to analyze the loci taught by McCamish using the combined method of Hardenbol and Mockler in order to ascertain whether a particular subject would be susceptible to drug therapy for blood glucose regulation.

Claim 8 lacks inventive step under PCT Article 33(3) as being obvious over Hardenbol, in view of Mockler, in further view of WO 2007/107717 A1 to Chamberlain et al. (hereinafter 'Chamberlain').

As per claim 8, the combination of Hardenbol and Mockler teaches the method of claim 7, but fails to specifically teach that which Chamberlain teaches, that is wherein the polymorphism is selected from Table 2 (Table 3, 4). As Chamberlain teaches that the listed SNP's are related to responsiveness to treatment with bisphosphonate (pg 4, ln 16-33), it would have been obvious to one skilled in the art to analyze the loci and polymorphisms as taught by Chamberlain using the combined method of Hardenbol and Mockler in order to determine who would be responsive to treatment with bisphosphonate.

-------Continued in Subsequent Supplemental Box------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 10/01293

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V.2: Citations and Explanations

Claims 17-18 meet the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Hardenbol teaches a a method of analyzing a plurality of genetic loci, the method comprising:
obtaining a plurality of molecular inversion probe capture products each comprising a molecular inversion probe and a target nucleic acid (pg 674, col 2, para 2; Fig. 2), wherein the sequence of the molecular inversion probe comprises a differentiator tag sequence and, optionally, a primer sequence (Fig. 2a), amplifying the plurality of molecular inversion probe capture products (pg 674, col 1, para 2), but fails to teach: determining numbers of occurrence of combinations of target nucleic acid and differentiator tag sequence of molecular inversion probe capture products in the amplified plurality, and if the number of occurrence of a specific combination of target nucleic acid sequence and differentiator tag sequence exceeds a predetermined value, detecting bias in the amplification of the molecular inversion probe comprising the specific combination. Therefore, claim 17 and claim 18, which depends therefrom, meet the aforementioned requirements for the above reasons.

Claim 57 meets the requirements set forth in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Hardenbol discloses a method comprising:
(i) contacting the target nucleic acid with a plurality of detection probes under conditions that permit hybridization of the detection probes to the target nucleic acid (pg 674, col 1, para 2), wherein each detection probe is a polynucleotide that comprises a first arm that hybridizes to a first region of the target nucleic acid and a second arm that hybridizes to a second region of the target nucleic acid, wherein the first and second regions are on a common strand of the target nucleic acid, and wherein the nucleotide sequence between the 5' end of the first region and the 3' end of the second region is the nucleotide sequence of a sub-target nucleic acid (Fig. 2a, b); and
(ii) capturing a plurality of sub-target nucleic acids that are hybridized with the plurality of detection probes (pg 674, col 1, para 3 to pg 674, col 2, para 1), but fails to specifically teach (iii) measuring the frequency of occurrence of a sub-target nucleic acid in the plurality of sub-target nucleic acids, wherein the frequency of occurrence of the subtarget nucleic acid in the plurality of sub-target nucleic acids is indicative of the length of the sub-target nucleic acid. Therefore claim 57 meets the aforementioned requirements for the above reasons.

Claims 1-14, 17, 18, 57, 66, have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference G0814.70000	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 10/01293	International filing date *(day/month/year)* 30 April 2010 (30.04.2010)	(Earliest) Priority Date *(day/month/year)* 30 April 2009 (30.04.2009)
Applicant GOOD START GENETICS		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/01293

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 15, 16, 19, 22-56, 64, 65, 67
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-8, and 57, directed to a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-14, 17, 18, 57 and 66

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/01293

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2010.01)
USPC - 436/94
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 436/94

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, Google Scholar
Search Terms:molecular inversion probe, multiplex, overlap, tile, stack, probe, DNA, bias, reduce, circular, padlock, genotyping, probe set

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y --- A	Hardenbol et al., 'Multiplexed genotyping with sequence-tagged molecular inversion probes' Nature Biotechnology, 2003, Vol. 21, No. 6, pages 673-678; abstract, Fig. 2; pg 673, col 2, para 2pg 674, col 1, para 2-3; pg 674, col 2, para 1-3; pg 677, col 1, para 3	66 ---- 1-8, 10-14 ---------- 17-18, 57
X --- Y	US 2003/0203370 A1 (YAKHINI et al.) 30 October 2003 (30.10.2003) abstract, para [0026]	9 ------ 10-14
Y	Mockler et al., 'Applications of DNa tiling arrays for while-genome analysis', Genomics, 2005, Vol. 85, pages 1-15; Fig. 2; pg 2, col 1, para 4 to pg 2, col 2, para 1	1-8
Y	US 2008/0280955 A1 (MCCAMISH et al), 13 Nov. 2008 (13.11.2008), Table 9, para [0258], claims 1, 15	6
Y	WO 2007/107717 A1 (CHAMBERLAIN et al) 27 Sep. 2007 (27.09.2007), Tables 3, 4; pg 4, ln 16-33	8
&	US 2010/0129896 A1 (KNAPP) 27 May 2010 (27.05.2010)	57

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 Oct. 2010 (15.10.2010)	28 OCT 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/01293

Continuation of Box III: Unity of Invention

Group II: claims 9-14, 17, 18, and 66, directed to a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject.

Group III: claims 20-21, directed to a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject.

Group IV: claims 58-63, directed to a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The inventions listed as Groups I - IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of analyzing a plurality of gene loci, comprising: contacting each of a plurality of target nucleic acids with a probe set, wherein the probe set comprises a plurality of probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids in the same strand of one of a plurality of subregions in the target nucleic acid, wherein the subregions of the target are different. The special technical feature of the Group II claims is a method of genotyping a subject, comprising determining in the sequence at least a threshold number of independently isolated nucleic acids that comprise target nucleic acid sequences and differentiator tag sequences, wherein the threshold number is a number of unique combinations of target nucleic acid and differentiator tag sequences that form unique combinations, and the target nucleic acid sequence is the sequence of a genomic locus of a subject. The special technical feature of the Group III claims is a method of determining whether a target nucleic acid has abnormal length, comprising evaluating the capture efficiency of a target nucleic acid in a sample from a subject. The special technical feature of the Group IV claims is a method of increasing the sensitivity of a nucleic acid detection assay comprising utilizing two different preparation methods on parallel samples and assaying the sequences of the samples, using the sequence information from both determinations to evaluate the genotype of a target nucleic acid.

The only common technical element shared by the above groups is that they are related to genotyping of nucleic acids in samples. This common technical element does not represent an improvement over the prior art of US 2001/0046673 A1 to French et al. (see abstract; para [0012]- wherein length of the target was abnormal due to insertion or deletion; para [0077] - allele specific hybridization using luminescence detection; para [0107] - DNA chips generally attempt to detect SNPs by measuring the extent of hybridization of DNA targets to DNA probes immobilized on the chip surface). Therefore, the inventions of Groups I - IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GSGE-009/01WO 29258/54	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2013/036575	International filing date *(day/month/year)* 15 April 2013	(Earliest) Priority Date *(day/month/year)* 16 April 2012
Applicant GOOD START GENETICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036575

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)
   - ☒ on paper
   - ☒ in electronic form

   b. (time)
   - ☐ in the international application as filed
   - ☐ together with the international application in electronic form
   - ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036575

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2013.01).
USPC - 435/6.11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12N 15/10, 15/11; C12P 19/34; C12Q 1/68; C40B 40/08 (2013.01)
USPC - 435/6.1, 6.11, 91.1; 506/2, 7, 16; 702/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC Class/Subclass(es): C12Q 1/6869, 1/6876, 1/6883, 1/701

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, Google, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2010/126614 A2 (PORRECA et al) 04 November 2010 (04.11.2010) entire document	1-6, 9-15, 18 --- 7, 8, 16, 17, 19, 20
Y	EP 1564306 A2 (MCGALL et al) 17 August 2005 (17.08.2005) entire document	7, 8, 16, 17, 19, 20
A	WO 2012/040387 A1 (MYLLYKANGAS et al) 29 March 2012 (29.03.2012) entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 2013	12 AUG 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	12 AUG 2013
Applicant's or agent's file reference GSGE-009/01WO 29258/54	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2013/036575	15 April 2013	16 April 2012

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	23 July 2013	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/036575

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☒ on paper

      ☒ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☒ subsequently to this Authority for the purposes of search

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/036575

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	3, 4, 7, 8, 12, 13, 16, 17	YES
	Claims	1, 2, 5, 6, 9-11, 14, 15, 18	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1, 2, 5, 6, 9-11, 14, 15 and 18 lack novelty under PCT Article 33(2) as being anticipated by Porreca et al. (hereafter Porreca).

Regarding Claim 1, Porreca discloses a method of improving performance of molecular inversion probe capture reactions (In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes, Pg. 32, Lns. 30-32; capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, Pg. 9, Lns. 15-16; methods include molecular inversion probe capture reactions, Pg. 22, Lns. 32-33), the method comprising: obtaining a nucleic acid (Invention provides a method of obtaining a nucleic acid preparation, Pg. 23, Lns. 18-19); fragmenting the nucleic acid into nucleic acid fragments (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12); and capturing a target on a nucleic acid fragment with a molecular inversion probe (Capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, Pg. 9, Lns. 15-16; DNA should be sheared into fragments, Pg. 77, Ln.12).

Regarding Claim 2, Porreca discloses the method of claim 1, further comprising the step of denaturing the nucleic acid or at least one nucleic acid fragment prior to the capturing step (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12; heat reaction to 95C for 5 min to denature genomic DNA, Pg. 75, Lns. 20-26 (nucleic acids are denatured prior to capture reaction); prior to performing a gene capture reaction, Pg. 44, Ln. 2).

Regarding Claim 5, Porreca discloses the method of claim 1, wherein the nucleic acid is selected from the group of genomic DNA, genomic RNA, whole or partial genome amplification product, high molecular weight DNA, and high molecular weight RNA (An experiment utilizing 1 ug of genomic DNA, Pg. 42, Ln. 20).

Regarding Claim 6, Porreca discloses the method of claim 1, wherein the step of fragmenting comprises mechanical fragmenting, chemical fragmenting, or enzymatic fragmenting (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12).

Regarding Claim 9, Porreca discloses the method of claim 2, wherein the step of denaturing comprises pH-based denaturing, heat-based deneturing, exonuclease degradation, or endonuclease nicking (Exonuclease digestion of non-circularized material is performed, Pg. 60, Ln. 21).

Regarding Claim 10, Porreca discloses a method of improving performance of capture reactions (In other embodiments, methods improve allelic representation in a capture pool and, thus, improve variant detection outcomes, Pg. 32, Lns. 30-32; capturing target nucleic acids from a genomic sample of the subject with molecular inversion probes, Pg. 9, Lns. 15-16; methods include molecular inversion probe capture reactions, Pg. 22, Lns. 32-33), the method comprising: obtaining a genomic nucleic acid (An experiment utilizing 1 ug of genomic DNA, Pg. 42, Ln. 20; invention provides a method of obtaining a nucleic acid preparation, Pg. 23, Lns. 18-19); fragmenting the genomic nucleic acid into nucleic acid fragments; contacting a probe to a nucleic acid fragment (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12; contacting a nucleic acid sample with a preparation of a probe e.g., a MIP [molecular inversion probe], Pg. 19, Ln.23), the probe comprising at least two target arms designed to hybridize upstream and downstream of a target sequence located on the nucleic acid fragment; and isolating the target sequence between the target arms; and analyzing the target sequence (Targeting arms of a probe that is designed to capture the target region or sequence thereof, Pg. 12, Lns. 11-12; both targeting arms may be designed to hybridize several bases or more upstream or downstream from the repeat region in such a way that the captured sequence includes a region of unique genomic sequence that on one or both sides of the repeat region; this unique region can then be used to identify the captured target e.g., based on sequence or hybridization information, Pg. 12, Lns. 20-24; associating unique sequence tags referred to as differentiator tag sequences with individual target molecules that are independently captured and/or analyzed, Pg. 39, Lns. 21-23).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/036575

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 11, Porreca discloses the method of claim 10, further comprising the step of denaturing the genomic nucleic acid or at least one nucleic acid fragment prior to the contacting step (Heat reaction to 95C for 5 min to denature genomic DNA (nucleic acids are denatured prior to contacting), Pg. 75, Ln. 25; prior to performing a gene capture reaction, Pg. 44, Ln. 2; wherein the target nucleic acid is a captured genomic locus, Pg. Ln. 3; analyzing a plurality of genetic loci are provided; the methods comprise contacting each of a plurality of target nucleic acids, Pg. 5, Lns. 26-28).

Regarding Claim 14, Porreca discloses the method of claim 10, wherein the nucleic acid is selected from the group of genomic DNA, genomic RNA and whole or partial genome amplification product (An experiment utilizing 1 ug of genomic DNA, Pg. 42, Ln. 20).

Regarding Claim 15, Porreca discloses the method of claim 10, wherein the step of fragmenting comprises mechanical fragmenting, chemical fragmenting, or enzymatic fragmenting (Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument; DNA should be sheared into fragments, Pg. 77, Lns. 11-12).

Regarding Claim 18, Porreca discloses the method of claim 10, wherein the step of denaturing comprises pH-based denaturing, heat-based denaturing, exonuclease degradation, or endonuclease nicking (Exonuclease digestion of non-circularized material is performed, Pg. 60, Ln. 21).

Claims 3, 4, 12, and 13 lack an inventive step under PCT Article 33(3) as being obvious over Porreca et al. (hereafter Porreca).

Regarding Claim 3, Porreca discloses the method of claim 1, but Porreca fails to explicitly disclose wherein the nucleic acid fragments are from about 5kb to about 100kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 5kb to about 100kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Regarding Claim 4, Porreca discloses the method of claim 1, wherein the nucleic acid fragments are about 1kb in length or more (Probes can be designed to capture targets [nucleic acids] having lengths in the range of up to 10, 20, 30.....1000, or more bps, [wherein 1000 base pair is equivalent to 1kb], Pg. 34, Lns. 7-8; DNA should be sheared into fragments, Pg. 77, Ln.12), but fails to explicitly disclose wherein the nucleic acid fragments are from about 1kb to about 10kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 1kb to about 10kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Regarding Claim 12, Porreca discloses the method of claim 10, but Porreca fails to explicitly disclose wherein the nucleic acid fragments are from about 5kb to about 100 kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 5kb to about 100kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Regarding Claim 13, Porreca discloses the method of claim 10, wherein the nucleic acid fragments are about 1kb in length or more (Probes can be designed to capture targets [nucleic acid] having lengths in the range of up to 10, 20, 30.....1000, or more bps, [wherein 1000 base pairs is equivalent to 1kb], Pg. 34, Lns. 7-8; DNA should be sheared into fragments, Pg. 77, Ln.12), but fails to explicitly disclose wherein the nucleic acid fragments are from about 1kb to about 10kb in length.
However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include nucleic acid fragments from about 1kb to about 10kb in length, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum range involves only routine skill in the art. The motivation for doing so would be to ensure that longer target regions and sub-regions of genomic DNA are captured by probes in order to analyze the full target.

Claims 7, 8, 16, 17, 19 and 20 lack an inventive step under PCT Article 33(3) as being obvious over Porreca et al. (hereafter Porreca) in view of McGall et al. (hereafter McGall).

Regarding Claim 7, Porreca discloses the method of claim 5, but Porreca fails to explicitly disclose wherein the chemical fragmenting comprises pH-based shearing.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches chemical fragmenting comprising pH-based shearing (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105°C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation of ds cDNA with Tris Buffer at high temperature, Para. [0085]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include chemical fragmenting comprising pH-based shearing as taught by McGall as preparation by fragmentation helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe (McGall, Para. [0020] and [0049]).

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 8, Porreca discloses the method of claim 6, but Porreca fails to explicitly disclose wherein the pH-based shearing also denatures the nucleic acid.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches pH-based shearing also denaturing the nucleic acid (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105° C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation mixtures containing 10 mg ds cDNA, 10 mM Tris-HCl, pH 7.2 at room temperature were incubated at 95°C for 75, 90, 105 and 120 minutes, Para. [0086], [wherein the instant application the use of pH or heat during the fragmenting step can result in denatured nucleic acid fragments, Pg. 11, Lns. 10-11]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include pH-based shearing also denaturing the nucleic acid as taught by McGall as DNA preparation by fragmentation and heat denaturation at 95°C helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe (McGall, Paras. [0020], [0049], [0086]).

Regarding Claim 16, Porreca discloses the method of claim 15, but Porreca fails to explicitly disclose wherein the chemical fragmentation comprises pH-based shearing.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches chemical fragmenting comprising pH-based shearing (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105°C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation of ds cDNA with Tris Buffer at high temperature, Para. [0085]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include chemical fragmenting comprising pH-based shearing as taught by McGall as preparation by fragmentation helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe, (McGall, Paras. [0020] and [0049]).

Regarding Claim 17, Porreca discloses the method of claim 14, but Porreca fails to explicitly disclose wherein the pH-based shearing also denatures the nucleic acid.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches pH-based shearing also denaturing the nucleic acid (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105° C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; fragmentation mixtures containing 10 mg ds cDNA, 10 mM Tris-HCl, pH 7.2 at room temperature were incubated at 95°C, Para. [0086], [wherein the instant application the use of pH or heat during the fragmenting step can result in denatured nucleic acid fragments, Pg. 11, Lns. 10-11]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include pH-based shearing also denaturing the nucleic acid as taught by McGall as DNA preparation by fragmentation and heat denaturation at 95°C helps to reduce the complexity of the nucleic acid sample and allows more efficient hybridization to a target probe, (McGall, Paras. [0020], [0049], [0086]).

Regarding Claim 19, Porreca discloses a method of preparing a library for sequencing (During a library preparation process, each nucleic acid molecule may be tagged with a unique differentiator tag sequence in a configuration that permits the differentiator tag sequence to be sequenced along with the target nucleic acid sequence of interest, Pg. 41, Lns. 3-5; methods for overcoming problems associated with systematic and random errors (bias) in genome capture, amplification and sequencing methods, Pg. 39, Lns. 6-8), the method comprising: amplifying a captured target nucleic acid to generate an amplicon (The target nucleic acid is a captured genomic locus of the subject, amplifying the molecular inversion probe capture products [nucleic acids], Pg. 9, Lns. 10-12; in the context of a multiplex nucleic acid amplification reaction, differentiator tag sequences provide a basis for differentiating between multiple independent, primary amplicons of a target nucleic acid, Pg. 40, Lns. 13-16), but Porreca fails to explicitly disclose performing a hydrolysis reaction on the amplicon in the presence of heat to fragment the amplicon.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches performing a hydrolysis reaction on the amplicon in the presence of heat to fragment the amplicon (The DNA sample to be fragmented is in an aqueous solution containing a buffer that is neutral (pH greater than or equal to 6.0) at a temperature between 20 and 37°C but becomes acidic (pH less than 6.0) at a temperature between 80 and 105°C. In one aspect the buffer is a Tris (Tris hydroyxmethyl aminomethane) buffer solution, Para. [0006]; DNA is fragmented by chemical or thermal treatment, Para. [0007]; amplifying the sample to generate amplified DNA [amplicon], Pg. 18, Ln. 6; fragmentation can be accomplished by altering temperature and pH, Pg. 7, Lns. 2-5; chemical methods for fragmentation of nucleic acids that are known in the art. These methods include: hydrolytic methods, Para. [0058]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include performing a hydrolysis reaction on the amplicon in the presence of heat to fragment the amplicon as taught by McGall as fragmentation methods including heat and hydrolytic methods can be used to select fragments of a desired size range and may be further desirable to optimize the size of nucleic acid molecules for subsequent analysis and minimize three dimensional structure, (McGall, Para. [0007], [0049] and [0058]).

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 20, modified Porreca discloses the method of claim 19, but Porreca fails to explicitly disclose wherein the step of performing the hydrolysis reaction comprises incubating the amplicon in a Tris buffer having a pH range from about 7.5 to about 8.0 at a temperature of about 65°C for 16 hours.
However, McGall is in the field of methods and compositions for fragmenting DNA (abstract) and teaches the step of performing the hydrolysis reaction comprising incubating the amplicon in a Tris buffer having a pH range from about 7.5 to about 8.0 at a temperature of about 65°C for 16 hours (Amplifying the sample to generate amplified DNA [amplicon], Pg. 18, Ln. 6; the DNA is in a solution that includes about 10 mM Tris-HCl, pH ~7.2-7.5, Para. [0060]; the DNA is mixed with the buffer at a temperature within the first temperature range and then incubated at a temperature in the second temperature range; the buffer is acidic in the second temperature range and the DNA is fragmented, Para. [0063]; in a preferred embodiment the buffer is Tris-HCl, Para. [0064]; tris or phosphate buffer pH 7-8.5 at 65°C is used to fragment single and double stranded DNA, Para. [0076]; the fragmentation reaction was incubated at 65°C, Para. [0097]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Porreca to include a step of performing the hydrolysis reaction comprising incubating the amplicon in a Tris buffer having a pH range from about 7.5 to about 8.0 at a temperature of about 65°C for 16 hours as taught by McGall as fragmentation using Tris buffer, heat at 65°C and hydrolytic methods can be used to select fragments of a desired size range and may be further desirable to optimize the size of nucleic acid molecules for subsequent analysis and minimize three dimensional structure (McGall, Para. [0007], [0049], [0058], [0064] and [0076]).

Claims 1-20 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GSGE-008/01WO 29258/45	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/55362	International filing date *(day/month/year)* 14 September 2012 (14.09.2012)	(Earliest) Priority Date *(day/month/year)* 17 October 2011 (17.10.2011)
Applicant GOOD START GENETICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

Methods of identifying mutations associated with diseases

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/55362

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-20, drawn to a method for identifying a novel mutation associated with a disease, the method comprising: obtaining nucleic acid from a subject having a disease; identifying at least one mutation in the nucleic acid; and comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations, as indicated in claims 1 or 11; or a method for determining if a mutation is causative of a disease, as indicated in claim 20. The first named invention (claims 1-5, 7-8, 11-14, 16-17) is limited to wherein identifying comprising: sequencing the nucleic acid; wherein sequencing is sequencing-by-synthesis, and wherein the disease is cystic fibrosis. **********Continued in the extra sheet****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 7-8, 11-14, 16-17, limited to wherein identifying comprising: sequencing the nucleic acid; wherein sequencing is sequencing-by-synthesis, and wherein the disease is cystic fibrosis

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/55362

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The disclosure relates to methods for analyzing nucleic acids to identify mutations associated with diseases. More specifically, the methods of the invention involve obtaining nucleic acid from a subject having a disease, identifying at least one mutation in the nucleic acid, and comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as newly identified mutations.

Form PCT/ISA/210 (continuation of first sheet (3)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/55362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11, 435/6.12, 435/6.17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12Q 1/68 (2013.01)
USPC - 435/6.11, 435/6.12, 435/6.17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C12Q 1/68 (2013.01) - see keyword below
USPC - 435/6.11, 435/6.12, 435/6.17 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: sequencing-by-synthesis, SBS, identifying, determine, screen, novel, mutation, new, disorder, disease, cancer, cystic fibrosis, mutation database, compare, sequencing-by-synthesis, single-molecule, obtain, sample, control, normal, Hispanic, nucleic acid, nucleotide, polynucleotide, DNA, RNA,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	CASTELLANI et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros. 2008, Vol. 7(3), p. 179-96. Abstract; pg 180, col 1, and col 2, lower para; pg 181, col 2, para 2; pg 182, col 2, para 1 and 3, and Table 2; pg 183, col 1, para 2, and col 2, para 1 and 2, and last para; pg 185, col 1, last para, and col 2, para 1; pg 186, col 1, para 3; pg 187, Table 5; pg 191, Table 7 and Table 8; pg 192, col 1, last para; and pg 193, col 1, Ref 4	1-2, 5, 7-8, 11,14, 16-17 --- 3-4, 12-13
Y	US 2011/0166029 A1 (MARGULIES et al.) 07 July 2011 (07.07.2011), para [0044], [0048], [0057], [0059], and [0064]	3-4, 12-13
A	SCHADT et al. A window into third-generation sequencing. Hum Mol Genet. 2010, Vol. 19(R2), p. R227-40. Epub 2010 Sep 21. pg R227, col 1, and col 2, middle para; pg R230, Table 1; and pg R231, col 1, last para	1-5, 7-8, 11-14, 16-17
A	NAN et al. A novel CFTR mutation found in a Chinese patient with cystic fibrosis. Comparing with normal database. Chinese Med J. 2006, Vol. 119(2), p. 103-109. Entire documentation, especially Abstract	1-5, 7-8, 11-14, 16-17
A	CHOE et al. Novel CFTR Mutations in a Korean Infant with Cystic Fibrosis and Pancreatic Insufficiency. J Korean Med Sci. 2010; Vol. 25, p. 163-5. Entire documentation, especially Abstract	1-5, 7-8, 11-14, 16-17
A	US 2011/0230365 A1 (ROHLFS et al.) 22 September 2011 (22.09.2011), Abstract, para [0005], [0071], [011], [0138], and [0150]	1-5, 7-8, 11-14, 16-17

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 February 2013 (07.02.2013)	25 FEB 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/55362

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/0192047 A1 (PARR et al.) 30 July 2009 (30.07.2009), para [0010], [0030], [0045], [0158], and [0200]	1-5, 7-8, 11-14, 16-17

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/55362

Continuation of:
Box No III (unity of invention is lacking)

(Continuation of Group I+) Applicant is invited to elect an additional features, selected from: 1) wherein prior to identifying, the method further comprises attaching a barcode sequence to the nucleic acid (claims 6 and 15); or/and 2) the method further comprising determining of the novel mutation is causative of the disease, or a method for determining if a mutation is causative of a disease, comprising: identifying at least one novel mutation in the sequence, annotating the mutation with appropriate functional information, as indicated in claim 20 (claims 9-10, 18-19, 20), to be searched by paying additional fee for each election. Further provided are exemplary elections: 1) if the an additional feature wherein the method further comprises attaching a barcode sequence to the nucleic acid (claims 6 and 15) is elected, claims 6 and 15 will be searched by paying an additional fee; 2) if the additional feature of the method further comprising determining of the novel mutation is causative of the disease, or a method for determining if a mutation is causative of a disease comprising identifying at least one novel mutation in the sequence, annotating the mutation with appropriate functional information (claims 9-10, 18-19, 20) is elected, claims 9-10, 18-20 will be searched by paying an additional fee; and 3) both additional features 1) and 2) are elected, all additional claims 6, 9-10, 15, and 18-20 will be searched by paying 2X additional fees.

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Among Group I+, claims 1-8 and 11-17 do not include the inventive concept of determining of the novel mutation is causative of the disease as required by the claims, annotating the mutation with appropriate functional information, as required by claims 9-10 and 18-20; and claims 1-5, 7-14, and 16-20 do not include the inventive concept of attaching a barcode sequence to the nucleic acid, as required by claims 6 and 15.

The inventions of Groups I+ share the technical feature of determining if a mutation associated with a disease, comprising: obtaining a nucleic acid sequence from a subject having a disease, identifying at least one mutation in the nucleic acid; and claims 1-19 of Group I+ further share the technical feature of identifying a novel mutation associated with a disease, the method comprising: obtaining nucleic acid from a subject having a disease; identifying at least one mutation in the nucleic acid; and comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations. However, this shared technical feature does not represent a contribution over prior art as being obvious over an article entitled 'Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice' by CASTELLANI et al. (hereinafter 'Castellani'; J Cyst Fibros. 2008, Vol. 7(3), p. 179-96) as follows:Castellani discloses a method for identifying a novel mutation associated with a disease (pg 183, col 1, para 2 ? 'Conclusion:...methods to detect mutations in the CFTR gene ... to extensive scanning/sequencing protocols that enable the detection of novel mutations', wherein 'novel mutations' 'in the CFTR gene' is 'associated with a disease' - 'cystic fibrosis (CF)'; pg 180, col 1 - 'cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene ... have resulted in the identification of more than 1500 CFTR mutations'; Abstract),
--- the method comprising: obtaining nucleic acid from a subject having a disease (pg 182, col 2, para 1 - 'identification of CFTR mutations in patients diagnosed with CF'; pg 180, col 1 - 'cystic fibrosis (CF)'; pg 191, Table 7 - 'Name of patient ... sample number'; pg 191, Table 8 - 'Molecular genetic analysis for cystic fibrosis.... Sample type: DNA')
---identifying at least one mutation in the nucleic acid (pg 183, col 2, para 2 - 'diagnosis of CF is based on a consistent phenotype plus evidence of CFTR channel dysfunction ... or identification of two CF-disease causing mutation in trans'; pg 183, col 2, last para - 'the term "mutation" is defined as a molecular alteration in the DNA sequence of a gene; pg 182, col 2, para 1 - 'identification of CFTR mutations in patients diagnosed with CF').
Although Castellani does not expressively teach comparing the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations, one of ordinary skill in the art at the time the invention was made would have known to do so, because it is the first step that one would have done to compare the mutation to a database of mutations in order to classify the mutation or to determine if the mutation identified is a novel mutation, especially Castellani further provides a database of mutations known to be associated with the disease, which is widely used by researchers in the field (pg 192, col 1, last para - 'The traditional mutation nomenclature is currently used in the vast majority of research papers, and in the CFGAC Mutation Database...we advise using the mutation names of the routinely and widely used CFTR mutation database [4]', wherein 'CFGAC Mutation Database' is 'a database of mutations known to be associated with the disease' - 'cystic fibrosis (CF)'; pg 181, col 2, para 2 - 'more than 1500 sequence alterations have been identified in the CFTR gene and their listing is continuously updated within the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) database [4]'; pg 193, col 1, References - '[4] Cystic Fibrosis Mutation Database. www.genet.sickkids.on.ca/cftr'); the classification of the mutations (pg 187, Table 5), as well as teaches criteria for characterization of novel mutations for determining the potential pathogenic potential of the mutation identified (pg 185, col 1, last para - 'Other criteria can be used to asses the pathogenic potential,..include: - the sequence variation causes a novel amino acid sequence ...the sequence variant changes a highly evolutionarily conserved amino acid residue...the sequence variation creates a novel/cryptic splice site...similar sequence variations are found in other ABC genes'). Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to compare the mutation to a database of mutations known to be associated with the disease, wherein mutations that do not match to the database are identified as novel mutations. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GSGE-007/01WO29258/52	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 13/33435	International filing date *(day/month/year)* 22 March 2013 (22.03.2013)	(Earliest) Priority Date *(day/month/year)* 04 April 2012 (04.04.2012)
Applicant GOOD START GENETICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/33435

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G06F 19/22 (2013.01)
USPC - 702/19, 20
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G06F 19/22 (2013.01)
USPC - 702/19, 20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (PGPB,USPT,USOC,EPAB,JPAB); PubMed (MEDLINE);
Search terms: contig, reads, alignment, insertion, deletion, mutation, reference, reference, mutation, polymorphism, SNP, identify, determine, assay, evaluate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0257889 A1 (KLAMMER et al.) 20 October 2011 (20.10.2011) para [0003], [0014], [0016], [0040], [0041], [0043], [0049], [0050], [0061], [0073], [0085], [0086], [0083]	1-6, 9-12 --- 7-8
Y	US 2009/0192047 A1 (PARR et al.) 30 July 2009 (30.07.2009) para [0236]; Table 4	7-8
X,P	US 8,209,130 B1 (KENNEDY et al.) 26 June 2012 (26.06.2012) entire document	1-12
A	KLEIN et al., LOCAS--a low coverage assembly tool for resequencing projects. PLoS One; 2011 (published online 15 August 2011); Vol 6, No 8, Document e23455; entire document	1-12
A	US 2004/0152108 A1 (KEITH et al.) 5 August 2004 (05.08.2004) entire document	1-12
A	US 2004/0142325 A1 (MINTZ et al.) 22 July 2004 (22.07.2004) entire document	1-12

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 May 2013 (03.05.2013)	10 JUN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	10 JUN 2013
Applicant's or agent's file reference: GSGE-007/01WO29258/52	FOR FURTHER ACTION: See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 13/33435	22 March 2013 (22.03.2013)	04 April 2012 (04.04.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06F 19/22 (2013.01)
USPC - 702/19, 20

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	03 May 2013 (03.05.2013)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 13/33435

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account **the rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRIT. OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

Inter nal application No.
PCT/US 13/33435

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	7-9	YES
	Claims	1-6, 10-12	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-6 and 10-12 lack novelty under PCT Article 33(2) as being anticipated by US 2011/0257889 A1 to Klammer et al. (hereinafter 'Klammer').

Regarding claim 1, Klammer discloses a method for identifying a mutation in a nucleic acid, the method comprising: sequencing nucleic acid to generate a plurality of sequence reads (para [0049]); creating a contig based on the reads (para [0016], [0040], [0050]); aligning the contig to a reference sequence (para [0017], [0043]); aligning the individual reads back to the contig (para [0050]); and identifying a mutation based on the alignments to the contig and the reference sequence (para [0043] - mutation that distinguishes the nucleotide sequence of the reference sequence).

Regarding claim 2, Klammer discloses wherein the method of claim 1 may further comprise splitting the reads into subsets corresponding to known locations (para [0083] - "overlap detection algorithms described above generally require a "query" and a "target," and so for de novo sequencing the sequence read dataset must provide both. In FIG. 5, the sequence dataset is provided to the algorithm as a plurality of subsets of the input sequence data set ("split data"), and these subsets can be used as the "query" or the "target" in a single pairwise comparison. In certain preferred embodiments, multiple of the subsets are combined to form a larger target for the comparison"; para [0086] - identifying locations of alignments between multiple occurrences of a known sequence within the sequencing read).

Regarding claim 3, Klammer discloses wherein the method of claim 1 may further comprise identifying a mutation in the contig relative to the reference (para [0043] - "loci that have basecalls that differ from the reference sequence can be further analyzed to determine if a given locus was erroneously called in the sequence read, or if it represents a true variation (e.g., a mutation, SNP variant, etc.)").

Regarding claim 4, Klammer discloses wherein the method of claim 1 may further comprise determining a position of the mutation in the nucleic acid based on a position of the mutation in the reads and the alignments to the contig and the reference sequence (para [0043] - "methods for aligning and assembling sequence reads based at least in part on a known reference sequence, which is sometimes termed "resequencing" or "mapping." In such embodiments, the sequence reads can be mapped to the reference sequence, and loci that have basecalls that differ from the reference sequence can be further analyzed to determine if a given locus was erroneously called in the sequence read, or if it represents a true variation (e.g., a mutation, SNP variant, etc.)"; para [0061]; the fourth position is mutated).

Regarding claim 5, Klammer discloses wherein the method of claim 1 may further comprise wherein the mutation may be selected from the group consisting of: a single nucleotide polymorphism (SNP), an insertion, a deletion, a substitution, and a translocation (para [0041], [0043]).

Regarding claim 6, further to the method of claim 1, Klammer discloses wherein a plurality of mutations may be identified (para [0014] - multiple deletion or insertions).

Regarding claim 10, further to the method of claim 1, Klammer discloses wherein the mutation may be associated with a disease (para [0003], [0004]).

Regarding claim 11, further to the method according to claim 1, Klammer discloses wherein sequencing may be sequencing-by-synthesis (para [0085]).

Regarding claim 12, further to the method according to claim 11, Klammer discloses wherein sequencing-by-synthesis may be a single molecule sequencing-by-synthesis (para [0038] - single-molecule real-time (SMRT.TM.) sequencing).

- Please see Supplemental Box for continuation -

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V.2: Citations and Explanations

Claim 9 lacks an inventive step under PCT Article 33(3) as being obvious over Klammer, as above.

Regarding claim 9, although Klammer does not expressly recite wherein the mutation is a deletion at an end of a sequence read, Klammer discloses wherein the mutation may be a deletion (para [0073]; k-mer ATGC may contain missing base, thereby producing deletion ATG). It would have been obvious to a person of ordinary skill in the art to anticipate wherein a mutation or errant base call may have been made at the end of a sequence read, or in a gap between sequence reads, due to a known tendency of polymerases to add bases beyond the end of a template, based on both the nucleotide mix available, and the preferences inherent in the particular polymerase.

Claims 7-8 lack inventive step under PCT Article 33(3) as being obvious over Klammer, as above, in view of US 2009/0192047 A1 to Parr et al. (hereinafter 'Parr').

Regarding claims 7-8, further to the method of claim 6, Klammer does not specifically teach the further limitations taught by Parr, wherein a first mutation may be within about 100 nucleotides of a second mutation, and wherein the first mutation may be a substitution and the second mutation may be a deletion (para [0236], Table 4). As Klammer further teaches wherein nucleotide sequence mutations may encompass multiple adjacent positions in the reference and/or sequencing reads (para [0043]), it would have been obvious to one having ordinary skill in the art that the method of claim 6 might further comprise wherein a first mutation may be within about 100 nucleotides of a second mutation, and wherein the first mutation may be a substitution and the second mutation may be a deletion, as taught by Parr.

Claims 1-12 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GSGE-011/01WO 29258/55	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 13/44039	International filing date *(day/month/year)* 04 June 2013 (04.06.2013)	(Earliest) Priority Date *(day/month/year)* 04 June 2012 (04.06.2012)
Applicant GOOD START GENETICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/44039

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G06F 19/22, C12Q 1/68 (2013.01)
USPC - 702/20, 435/6.11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): G06F 19/22, C12Q 1/68 (2013.01)
USPC: 702/20, 435/6.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 702/19, 435/6.12, 536/23.1
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms used: clinical significance, variant sequence, sequence read, reference sequence, genetic profile, sequencing-by-synthesis, single molecule sequencing, equivalent indel region, insertion or deletion, tandem repeat, splice site frameshift mutation, functional region, DNA RNA eDNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0166029 A1 (MARGULIES et al.) 7 July 2011 (07.07.2011) para [0010], [0011], [0046], [0048], [0049], [0067], [0078], [0081], [0088], [0095], [0096], [0101], [0104]	1-6, 8, 9 ---------- 7, 10
Y	US 2009/0129647 A1 (DIMITROVA et al.) 21 May 2009 (21.05.2009) Abstract, para [0040]	7
Y	WO 2012/051208 A2 (NAZARENKO etal.) 19 April 2012 (19.04.2012) para [0007], [0009], [0040], [0041]	10

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2013 (14.10.2013)	01 NOV 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	01 NOV 2013

Applicant's or agent's file reference	FOR FURTHER ACTION
GSGE-011/01WO 29258/55	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 13/44039	04 June 2013 (04.06.2013)	04 June 2012 (04.06.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06F 19/22, C12Q 1/68 (2013.01)
USPC - 702/20, 435/6.11

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	14 October 2013 (14.10.2013)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/44039

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/44039

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	7, 10	YES
	Claims	1-6, 8, 9	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-10	NO
Industrial applicability (IA)	Claims	1-10	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-6, 8 and 9 lack novelty under PCT Article 33(2) as being anticipated by US 2011/0166029 A1 to Margulies et al. (hereinafter 'Margulies').

Regarding claim 1, Margulies discloses a method for determining the clinical significance of a variant sequence (para [0010] "The method may comprise the steps of obtaining a nucleic acid ... from a subject and conducting an assay to identify whether there is a variant sequence in the subject's nucleic acid.", para [0011] "the invention may comprise methods for identifying mutations (i.e., variants) correlated with the presence or increased risk of developing an autism spectrum disorder.", para [0078] "The method may further include the steps of assessing the clinical significance of a variant or variants in the nucleic acid sequence ... for autism spectrum disorders."),
the method comprising: sequencing a nucleic acid to generate at least one sequence read (para [0046] "In some embodiments, the method may comprise obtaining a nucleic acid ... from a subject and sequencing at least a portion of a nucleic acid in order to obtain a sample nucleic acid sequence for at least one gene.");
identifying a variant sequence within the sequence read based on mapping the sequence read to a reference sequence (para [0081] "The nucleic acid may be sequenced ... in order to detect variants (i.e., mutations) in the nucleic acid compared to a reference sequence derived from one or more individuals not known to suffer from an autism spectrum disorder", para [0088] "In next generation (massively parallel sequencing), all regions of interest are sequenced together, and the origin of each sequence read is determined by comparison (alignment) to a reference sequence.");
determining the equivalent indel [i. e. insertion or deletion] region of the variant sequence (para [0101] "types of variants include deletions of one or more nucleotides ... insertions of one or more nucleotides");
identifying a functional region comprising at least a portion of the equivalent insertion/deletion region (para [0101] "As shown in Table 2, sequence variants are named according to the change they cause in the DNA sequence and the resultant change (if any) to the peptide sequence.", para [0104] "Frameshift mutations cause a shift in reading frame leading to a complete change of the amino acid sequence downstream of the mutation (i.e., the frameshift site). A frameshift mutation is caused by a net deletion or insertion of a number of nucleotides not divisible by 3."),
associating the equivalent indel region with the identified functional region, thereby to determine clinical significance of the variant (para [0101] "types of variants include deletions of one or more nucleotides ... insertions of one or more nucleotides", para [0095] "As depicted in FIG. 2, the variants and/or variant combinations may be assessed for their clinical significance for autism spectrum disorders based on one or more of the following methods. If a variant or a variant combination is reported or known to occur more often in nucleic acid from subjects with, than in subjects without, autism spectrum disorders, it is considered to be at least potentially predisposing to autism spectrum disorders. ... Conversely, if a variant is found in both populations at a similar frequency, it is less likely to be associated with the development of an autism syndrome disorder (ASD)", para [0096] " if a variant or a variant combination is predicted to have an overall deleterious effect on a protein or gene expression (i.e., resulting in a nonsense mutation, a frameshift mutation, or a splice site mutation ...), based on the predicted effect on the sequence and/or the structure of a protein or a nucleic acid ... it is considered to be at least potentially predisposing to autism spectrum disorders").

Regarding claim 2, Margulies further teaches nucleic acid that is DNA and RNA (para [0067] "The techniques for sequencing nucleic acids (both DNA and RNA) are highly sensitive and therefore, can be used almost any biological sample (i.e., tissue or body fluid) taken from subject.").

Regarding claim 3, Margulies further teaches sequencing that is sequencing-by-synthesis (para [0048] "In various embodiments of the methods of the invention ... the assay comprises ... nucleic acid sequencing ... Additionally and/or alternatively, the sequencing comprises at least one of single-molecule sequencing-by-synthesis.").

----------SEE FIRST SUPPLEMENTAL BOX TO CONTINUE----------

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V No. (2) Citations and Explanations:

Regarding claim 4, Margulies further teaches sequencing that is single molecule sequencing (para [0081] "The nucleic acid may be sequenced ... in order to detect variants (i.e., mutations) in the nucleic acid compared to a reference sequence ... sequence variants ... can be detected by any sequencing method known in the art e.g., ... single molecule sequencing").

Regarding claim 5, Margulies further teaches variant sequences that comprise a genetic mutation (para [0081] "The nucleic acid may be sequenced and/or its genomic arrangement and/or copy number is determined in order to detect variants (i.e., mutations) in the nucleic acid compared to a reference sequence derived from one or more individuals not known to suffer from an autism spectrum disorder").

Regarding claim 6, Margulies further teaches genetic mutations that are an insertion or a deletion (para [0101] "types of variants include deletions of one or more nucleotides ... insertions of one or more nucleotides", para [0104] "Frameshift mutations cause a shift in reading frame leading to a complete change of the amino acid sequence downstream of the mutation (i.e., the frameshift site). A frameshift mutation is caused by a net deletion or insertion of a number of nucleotides not divisible by 3.");

Regarding claim 8, Margulies further teaches determining a functional region that comprises a gene, an exon, an intron, a splice site, a regulatory element (para [0010] "The method may comprise the steps of obtaining a nucleic acid ... and conducting an assay to identify whether there is a variant sequence in the ... nucleic acid.", para [0049] "the nucleic acid in the conducting step comprises a gene ... an exon, an intron, a gene regulatory element ... including splice sites ... may be evaluated for mutations (i.e., variants).").

Regarding claim 9, Margulies further teaches variant sequences is a splice site mutation, a nonsense mutation, or a frameshift mutation (para [0096] "if a variant or a variant combination is predicted to have an overall deleterious effect on a protein or gene expression (i.e., resulting in a nonsense mutation, a frameshift mutation, or a splice site mutation, or even a missense mutation), based on the predicted effect on the sequence and/or the structure of a protein or a nucleic acid, and if this variant or variant combination affects a gene or genes known to be associated with autism spectrum disorders, it is considered to be at least potentially predisposing to autism spectrum disorders").

Claim 7 lacks an inventive step under PCT Article 33(3) as being obvious over Margulies in view of US 2009/0129647 A1 to Dimitrova (hereinafter 'Dimitrova').

Regarding claim 7, Margulies does not expressly teach identifying a genetic mutation that is located in a tandem repeat, however, Dimitrova teaches methods for said identifying (Abstract "Spectrogram extraction from DNA sequence has been known since 2001. ...Given a DNA sequence or whole genomes, with this method it is easy to generate a large number of spectrogram images. However, the difficult part is to elucidate where are the repetitive patterns and to associate a biological and clinical meaning to them. The present disclosure provides systems and methods that facilitate the location and/or identification of repetitive DNA patterns, such as ..tandem repeats", para [0040] "As described herein, exemplary systems and methods support and/or utilize one or more of the following DNA spectral analysis techniques: (i) comparative histogram methodologies; (ii) selection/classification using support vector machines and genetic algorithms; and (iii) spectrovideo methodologies based on spectrogram extractions from DNA sequence data. Many benefits may be realized according to the present disclosure, e.g., (i) enhanced visualization of genomic information, (ii) identification of repetitive DNA patterns, e.g. .. tandem repeats ...to permit rapid visualization of an entire genome at a desired resolution.). It would have been obvious to one of ordinary skill in the art to add Dimitrova's methods for identifying tandem repeat mutations among mutations in a nucleic acid, because said mutations are difficult to characterize, as taught by Dimitrova, but may add new clinically significant mutations to those identified by the method of Margulies.

Claim 10 lacks an inventive step under PCT Article 33(3) as being obvious over Margulies in view of WO 2012/051208 A2 to Nazarenko et al. (hereinafter 'Nazarenko').

Regarding claim 10, Margulies does not expressly teach determining whether the equivalent indel region extends beyond the functional region, however, Nazarenko teaches said determination (para [0007] "Embodiments of the present invention can provide identification of junctions (e.g., resulting from large-scale rearrangements) of a sequenced genome with respect to a human genome reference sequence.", para [0009] "a method is provided for determining whether a clinically significant junction exists between a sample genome and a reference genome", para [0040] "To determine a genome of an organism, fragments from a biological sample can have their two ends sequenced ... These mated pairs of sequence reads can then be mapped to one or more reference genomes to determine the sample genome. ... in some cases, pairs cannot be mapped as expected to a reference genome, and are called discordant pairs. ... Discordant mate pairs can occur when a rearrangement, or a large insertion or deletion, has occurred in the sample genome relative to the reference genome.", para [0041] "The accurate identification of rearrangement locations is important as such cases typically are the result of disease, such as cancer, ... Such rearrangement can include, for example ... a piece of the genome being deleted. Such rearrangements can cause the loss of a gene, a function of the gene, and a protein created from the gene."). It would have been obvious to one of ordinary skill in the art to combine Nazarenko's method of determining whether the equivalent indel region extends beyond the functional region with Margulies' method for determining the clinical significance of a variant sequence having smaller or uncharacterized indel mutations, because said determining could reveal other clinically significant aspects of extended indel regions, as taught by Nazarenko (para [0041] "since there are changes in which portions of the genome become near each other, a new expression or disregulation can occur. ... the discovery of larger insertions, deletions, duplications, and rearrangements can add value beyond the discovery of small variations.").

Claims 1-10 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GSGE-014/01 WO 29258/64	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US13/62842	International filing date *(day/month/year)* 01 October 2013 (01.10.2013)	(Earliest) Priority Date *(day/month/year)* 07 November 2012 (07.11.2012)
Applicant Good Start Genetics, Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☐ as suggested by the applicant.

   ☒ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/62842

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/62842

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G06N 3/12, 7/02; G06F 17/10 (2013.01)
USPC - 706/13, 21; 702/19
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): G06N 3/12, 7/02; G06F 3/00, 17/00, 17/10, 15/18; A61B 5/00, 5/07; C40B 40/10, 40/00, 60/10; A61N 1/30; C12Q 1/68 (2013.01)
USPC Classification(s): 706/13, 21, 52, 15; 702/19; 703/1; 424/422; 600/300, 483; 506/13, 18, 38, 7; 250/282

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-B, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google/Google Scholar; ScienceDirect; ProQuest; Search Terms: genetic, mutation, test, evaluate, computer, sequence, pipeline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LI, M et al. A New Approach for Detecting Low-level Mutations in Next-generation Seqence Data. Genome Biology. 23 May 2012, Vol. 13, pp. 1-15; page 4, left column, second paragraph; page 12 right column, second paragraph. DOI: 10.1186/gb-2012-13-5-r34	1-11
Y	US 2012/0252684 A1 (SELIFONOV, S et al.) October 4, 2012; abstract; paragraphs [0027], [0080], [0138], [0139], [0147].	1-11
Y	US 8209130 B1 (KENNEDY, C et al.) June 26, 2012; abstract; column 2, lines 20-21; column 2, lines 44-45; column 3, lines 18-24; column 3, lines 63-64; column 4, lines 1-5; column 5, lines 48-52	6-8, 10, 11
Y	EP 1321477 B1 (GRAWUNDER, U et al.) October 13, 2004; page 5, lines 24-27; page 17, lines 32-33	4

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 December 2013 (30.12.2013)	04 FEB 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Thomas C Meyers
Brown Rudnick LLP
One Financial Center
Boston, MA 02111
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	04 FEB 2014

Applicant's or agent's file reference	FOR FURTHER ACTION
GSGE-014/01 WO 29258/64	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US13/62842	01 October 2013 (01.10.2013)	07 November 2012 (07.11.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G06N 3/12, 7/02; G06F 17/10 (2013.01)
USPC - 706/13, 21; 702/19

Applicant Good Start Genetics, Inc.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	30 December 2013 (30.12.2013)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/62842

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☒ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US13/62842

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-11	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-11	NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-3, 5, and 9 lack an inventive step under PCT Article 33(3) as being obvious over the article entitled 'A new approach for detecting low-level mutations in next-generation sequence data' by Li, et al. (hereinafter 'Li') in view of US 2012/0252684 A1 to Selifonov, et al. (hereinafter 'Selifonov').

As per claim 1, Li discloses a method of evaluating a genetic test (we developed a new method that distinguishes real mutations (genetic test) from sequencing errors and test it using three different distributions to evaluate the performance of our method compared to two others; page 4, left column, second paragraph), the method comprising: obtaining, using a computer (here we provided a novel computation method (on a computer system), all common variation positions were flagged in the output file; page 12, right column, second paragraph; supplemental page 5, second paragraph), a plurality of sequence reads (for each bin at a specific position we counted the number of reads with major and minor alleles for the individual (individual sequence) in question as well as the same numbers for the reference individuals (plurality of sequences); supplemental page 3, second paragraph); analyzing the sequence reads using a genetic pipeline (all samples (sequence reads) are analyzed following the same pipeline; page 11, left column, second paragraph); determining if the genetic pipeline identified the mutation (figure 4 shows the low level mutations and sequencing errors detected by the genetic pipeline; page 5, figure 4), thereby validating the genetic pipeline (our method (genetic pipeline) showed a better performance because the empirical error rate is low, making it easier to distinguish real low-level mutations from sequencing errors (simulated mutations); page 8, right column, third paragraph). Li does not disclose a computer comprising a non-transitory memory; introducing, using the computer, a simulated mutation into at least one of the plurality of sequence reads. Selifonov discloses a computer comprising a non-transitory memory (the data structure is designed for a computer can be formed in computer memory such read-only memory (non-transitory memory); paragraph [0147]); introducing, using the computer (the computer program comprises computer code that performs the operations described herein; paragraph [0020]), a simulated mutation into at least one of the plurality of sequence reads (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) the mutations can be random or can be placed into a specific subunit; paragraphs [0027], [0138], [0139]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

As per claim 2, Li and Selifonov, in combination, disclose the method of claim 1, and Li further discloses introducing artificial DNA into about half of the plurality of sequence reads (artificially mixing of 2 DNA samples was done at a ratio of 1:1; page 13, left column, second paragraph). Li does not disclose introducing the simulation sequence. Selifonov discloses introducing the simulation sequence (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) the mutations can be random or can be placed into a specific subunit; paragraphs [0027], [0138], [0139]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

As per claim 3, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose wherein introducing the simulated mutation comprises manipulating a data field for base sequence or quality. Selifonov discloses wherein introducing the simulated mutation comprises manipulating a data field for base sequence (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) the mutations can be random or can be placed into a specific subunit, the data structure can be manipulated by an appropriate computer; paragraphs [0027], [0138], [0139], [0147]) or quality. It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

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Supplemental Box

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-***-Continued from Box V: Citations and Explanations-***-

As per claim 5, Li and Selifonov, in combination, disclose the method of claim 1, and Li further discloses assembling the sequence reads (page 2, Figure 1) Li does not disclose the method comprising assembling the sequence reads prior to introducing the simulated mutation. Selifonov discloses the method comprising assembling the sequence reads prior to introducing the simulated mutation (the variation (mutation) can be introduced in to the concatenated string output (after assembling has occurred); paragraphs [0138], [0182], [0183]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

As per claim 9, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose using the computer to automatically introduce a number of different simulated mutations into different ones of the plurality of sequence reads. Selifonov discloses using the computer (the computer program comprises computer code that performs the operations described herein; paragraph [0020]) to automatically introduce a number of different simulated mutations into different ones of the plurality of sequence reads (the methods of this invention can be used to introduce variation into the initial strings (plurality of sequence reads) automatically using a computer, the mutations can be random or can be placed into a specific subunit; paragraphs [0027], [0080], [0138], [0139]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the computer induced mutations, as previously disclosed by Selifonov, as the previous disclosure by Li includes the use of known mutations to test and verify next generation sequencing technologies in order to improve discovery of genetic variation in populations, Selifonov discloses a method for increasing genetic variation in a population by introducing simulated mutations (Selifonov; abstract), and a combination of these references would have resulted in a superior method of evaluating a genetic test.

Claims 6-8, 10, and 11 lack an inventive step under PCT Article 33(3) as being obvious over Li in view of Selifonov, in further view of US 8,209,130 B1 to Kennedy, et al. (hereinafter 'Kennedy').

As per claim 6, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose wherein the analyzing step comprises assembling the plurality of sequence reads to produce a contig. Kennedy discloses wherein the analyzing step comprises assembling the plurality of sequence reads to produce a contig (nucleic acid obtained from a subject is sequenced to produce a plurality of reads and the methods of the invention are accomplished by assembling contigs from sequence reads; column 2, lines 20-21; column 2, lines 44-45). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would have produced a contig that could be compared to a reference sequence.

As per claim 7, Li, Selifonov, and Kennedy, in combination, disclose the method of claim 6, but Li does not disclose wherein the analyzing step further comprises aligning the contig to a reference. Kennedy discloses wherein the analyzing step further comprises aligning the contig to a reference (mutations are identified based on the alignments to the reference and to the contig; abstract). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would have produced a contig that could be compared to a reference sequence.

As per claim 8, Li, Selifonov, and Kennedy, in combination, disclose the method of claim 7, but Li does not disclose wherein the analyzing step further comprises aligning one or more of the plurality of sequence reads to the contig. Kennedy discloses wherein the analyzing step further comprises aligning one or more of the plurality of sequence reads to the contig (with the contigs aligned, each raw read is aligned to a contig in a local alignment, all reads (plurality) can align as a perfect match; figures 1, 2; column 4, lines 1-2; column 4, lines 4-5). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the alignment method, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would be capable of identifying mutations.

As per claim 10, Li and Selifonov, in combination, disclose the method of claim 1, and Li further discloses the genetic pipeline (our method (genetic pipeline) showed a better performance because the empirical error rate is low, making it easier to distinguish real low-level mutations from sequencing errors (simulated mutations); page 8, right column, third paragraph). Li does not disclose comprising instructions in the memory operable to cause the computer to assemble the plurality of sequence reads. Kennedy discloses instructions in the memory operable to cause the computer to assemble the plurality of sequence reads (using barcodes reads (plurality) can be sorted using a computer processor (memory), then assembled into contigs based on the barcodes; figures 1, 2, 3; column 3, lines 18-24). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstract), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would be capable of identifying mutations.

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Supplemental Box

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As per claim 11, Li, Selifonov, and Kennedy, in combination, disclose the method of claim 10, but Li does not disclose wherein the computer is operable to detect a mutation and report the mutation in relation to a reference. Kennedy discloses wherein the computer is operable to detect a mutation and report the mutation in relation to a reference (each mutation identified here can be described with reference to the reference genome using a computer; figures 1, 2, 3; column 3, lines 63-64). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of evaluating a genetic test, as previously disclosed by Li, with the assembly of sequence reads to produce contigs, as previously described by Kennedy, as the previous disclosure by Li includes identifying low-level mutations in sequences, and the Kennedy reference includes identifying mutations based on alignments to references (Kennedy; abstrac), and the combination of these references would have resulted in the use of a superior method to evaluate a genetic test that would be capable of identifying mutations.

Claim 4 lacks an inventive step under PCT Article 33(3) as being obvious over Li and Selifonov and in further view of EP 1,321,477 B1 to Grawunder, et al. (hereinafter 'Grawunder').

As per claim 4, Li and Selifonov, in combination, disclose the method of claim 1, but Li does not disclose wherein the simulated mutation comprises two mutations in cis. Grawunder discloses wherein the simulated mutation comprises two mutations in cis (in order to use the selected cells for antibody production it must be prevented that further productive endogenous Ig gene rearrangements can be performed in these cells, and this can be done by mutating cis on both alleles of the IgH and IgL (two mutations); page 5 lines 24-27). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method for evaluating a genetic test, as previously disclosed by Li, with the cis mutations, as previously described by Grawunder, as the previous disclosure by Li includes the testing of a DNA library (Li; page, 10, right column, third paragraph) and the Grawunder reference includes the use of exons encoding variable domains derived from a library of diverse arrangements (Grawunder; page 17, lines 32-33), and the combination of these references would have resulted in the use of a simulated mutation comprising two mutations in cis that would allow for better detection of low-frequency mutations such as cis mutations.

Claims 1-11 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

Electronic Acknowledgement Receipt

EFS ID:	19246222
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	09-JUN-2014
Filing Date:	07-APR-2011
Time Stamp:	17:07:18
Application Type:	Utility under 35 USC 111(a)

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Submitted with Payment	no

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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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9	Non Patent Literature	Benner_TrendsinGenetics_2001.PDF	530901 3557d643d22f6d8724bf107825e29d22f0d29986	no	5
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	19246524
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	09-JUN-2014
Filing Date:	07-APR-2011
Time Stamp:	17:08:48
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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2	Non Patent Literature	JaijoInvestOphtham2010.PDF	1767653 b7268e89aca5aea064fa645615e5ca3fe308ef3f	no	7
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13	Non Patent Literature	LecompteGene2001.PDF	2147927 94dce250c2eb9f5542b6fd36c07fa562ac08074f	no	14
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23	Non Patent Literature	Marras-1999-GenAnaly.PDF	364179 f03a0ca474e405e8e0d5d678edda316567f5b238	no	6
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26	Non Patent Literature	Mills-2010-Nature.PDF	857168 54f1d13a369a39f614a652f047ff8256bd960dff	no	7
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29	Non Patent Literature	Moudrianakis-1965-PNAS.PDF	1197431 d0cd6afbddfba3328d552a257c5ce55f96503672	no	8
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30	Non Patent Literature	Mullan.PDF	207371 94c553451f59a2ace31ac782f02543697a07e1b4	no	3
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36	Non Patent Literature	NilssonTrendsinBiotech2006.PDF	4780783 6a1214d422e49dc860d3c3aa57cb56e3bfef2255	no	6
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37	Non Patent Literature	NingScienceGenomeResearch2001.PDF	112487 81a8606bef710fa84edc9f9fba9bb603e59969a8	no	5
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38	Non Patent Literature	Nordhoff2003Nucleic_Acids_Research.PDF	814372 93702861b4d162d965e811b59e09f20d3d085d0b	no	11
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39	Non Patent Literature	OefnerNucleicAcidsResearch1996.PDF	11481773 4b2da0bf66ad5d2d2f7b4b7d321a4305256e3af3	no	8
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47	Non Patent Literature	Pieles1993NuclAcidsRes.PDF	951230 1af11705a923636fdcc6c94c2bbd52b8b3b63b29	no	6
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51	Non Patent Literature	Richter-2008-PloSOne.PDF	844357 de57d36c828be1500b8a5829a2adfe2561c86455	no	12
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52	Non Patent Literature	RobertsNucleicAcidsResearch1980.PDF	601283 500be52ebfc3cb468d7ab5f30070dfd3d3a5b22e	no	19
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56	Non Patent Literature	SangerPNAS1977.PDF	2920942 70fc0ddc32cf475e8b0f091c7fff97f1964dcece	no	5
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			Total Files Size (in bytes):	154654613	

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	13081660	Filing Date	2011-04-07	Docket Number (if applicable)	GSGE-001/01US	Art Unit	1637
First Named Inventor	Gregory Porreca			Examiner Name	T. E. Strzelecka		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

☐ Other _____

☒ Enclosed

☒ Amendment/Reply

☒ Information Disclosure Statement (IDS)

☐ Affidavit(s)/ Declaration(s)

☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 50-0369

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

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PTO/SB/30EFS (07-09)
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Signature of Registered U.S. Patent Practitioner			
Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2014-06-09
Name	Thomas C. Meyers	Registration Number	36989

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Porreca et al.	ART UNIT:	1637
SERIAL NUMBER:	13/081,660	CONFIRMATION NO.:	4441
FILING DATE:	April 7, 2011	EXAMINER:	Strzelecka, Teresa E.
TITLE:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION		

FILED ELECTRONICALLY

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE

This paper is in response to a final Office Action mailed March 7, 2014 from the United States Patent and Trademark Office. This response is filed with a Request for Continued Examination (RCE). Applicants authorize the Commissioner to charge the extension fee and any other fees due to Deposit Account No. 500369.

Amendments to the specification begin on page 2.

Amendments to the claims begin on page 3.

Remarks begin on page 10.

Amendments to the specification

No amendments to the specification are made herein.

Amendment to the claims

Please amend claims 1, 20, and 35 and cancel claims 5-6, 33-34, and 39-40 as follows. This list of claims replaces all prior versions and listings of claims in this application.

1. (Currently amended) A method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction, the method comprising:

obtaining a template nucleic acid;

incorporating at least two of the same sequence identifiers into the template;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the two incorporated sequence identifiers remain associated with the template, thereby validating the integrity of the nucleic acid template in a multiplex sequencing reaction.

2. (Original) The method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

3. (Original) The method according to claim 2, wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

4. (Withdrawn) The method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

5-6. (Canceled)

7. (Original) The method according to claim 1, wherein prior to sequencing, the template is attached to a substrate.

8. (Original) The method according to claim 2, wherein the template is directly attached to the substrate.

9. (Withdrawn) The method according to claim 2, wherein the template is indirectly attached to the substrate.

10. (Original) The method according to claim 7, further comprising, amplifying the template.

11. (Original) The method according to claim 1, wherein sequencing is sequencing by synthesis.

12. (Original) The method according to claim 11, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

13. (Original) The method according to claim 1, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

14. (Original) The method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

15. (Original) The method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

16. (Original) The method according to claim 1, wherein the pair of identifiers are first and second barcode sequences that are attached to the template.

17. (Withdrawn) The method according to claim 16, wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template.

18. (Original) The method according to claim 17, wherein the first and second barcode sequences are the same.

19. (Withdrawn) The method according to claim 17, wherein the first and second barcode sequences are different.

20. (Currently amended) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a pair of the same barcode sequences to the template;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

21. (Previously presented) The method according to claim 20, wherein a first barcode sequence is incorporated into a 5' portion of the template and a second barcode sequence is incorporated into a 3' portion of the template.

22. (Original) The method according to claim 21, wherein after attaching of the pair of barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

23. (Withdrawn) The method according to claim 20, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

24. (Original) The method according to claim 20, wherein prior to sequencing, the template is attached to a substrate.

25. (Original) The method according to claim 24, wherein the template is directly attached to the substrate.

26. (Withdrawn) The method according to claim 24, wherein the template is indirectly attached to the substrate.

27. (Original) The method according to claim 24, further comprising, amplifying the template.

28. (Original) The method according to claim 20, wherein sequencing is sequencing by synthesis.

29. (Original) The method according to claim 28, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

30. (Original) The method according to claim 20, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

31. (Previously presented) The method according to claim 30, wherein the labeled nucleotide is an optically labeled nucleotide.

32. (Original) The method according to claim 31, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

33-34. (Canceled)

35. (Currently amended) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template, wherein the first and second barcode sequences are the same;

amplifying the template;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

36. (Original) The method according to claim 35, wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

37. (Withdrawn) The method according to claim 35, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

38. (Original) The method according to claim 35, wherein prior to amplifying, the template is immobilized to a solid support.

39-40. (Canceled)

41. (Original) The method according to claim 35, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

42. (Withdrawn) A method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte.

43. (Withdrawn) The method of claim 42, wherein said identifiers are nucleic acid sequence tags.

44. (Withdrawn) The method of claim 43, wherein said sequence tags are associated with a moiety that binds said analyte.

45. (Withdrawn) The method of claim 42, wherein said analyte is a protein.

46. (Withdrawn) The method of claim 45, wherein said identifiers are peptides.

Remarks

Claims 1, 20, and 35 are amended to recite that at least two of the same sequence identifiers are incorporated into a nucleic acid template. Support for that amendment is found in claims 5, 33, and 39, which claims are canceled along with claims 6, 34, and 40. No new matter is added.

Rejection Under 35 U.S.C. §102

The Office Action rejects claims 1-3, 7-8, 10-12, 16, 20-22, 24-25, 27-29, 35-36, and 38 under 35 U.S.C. §102(b) in view of Parameswaran (Nucl. Acids. Res., 35:e130, pp. 1-9, 2007). Without acquiescing to the rejection and in order to advance the case to allowability, claim 1 is amended to incorporate the subject matter of claim 5, claim 20 is amended to incorporate the subject matter of claim 33, and claim 35 is amended to incorporate the subject matter of claim 39. Claims 5, 33, and 39 are not rejected in view of Parameswaran and are therefore the Patent Office considers those claims novel in view of Parameswaran. Since the subject matter of those claims has been incorporated into claims 1, 20, and 35, amended claims 1, 20, and 35 are novel in view of Parameswaran. For at least those amendments and reasons, Applicants respectfully request withdrawal of the 35 U.S.C. §102 rejection.

Rejection Under 35 U.S.C. §103(a)

The Office Action rejects claims 1-3, 5, 7-8, 10-16, 20-22, 24-25, 27-32, 35-36, 38-39, and 41 under 35 U.S.C. §103(a) in view of the combination of Kawashima (U.S. patent application publication number 2005/0100900), Parameswaran, and Kircher et al. (Bioessays, 32:524-536, 2010). Applicants respectfully disagree.

In order to establish a prima facie case of obviousness, three basic criteria must be established by the Patent Office. First, there must be a suggestion in the cited art to modify a reference or to combine multiple references in a manner that would lead one of ordinary skill in the art to an applicant's claimed invention. Second, the cited reference itself must convey a

reasonable expectation of success in arriving at the claimed invention based upon the proposed modification or combination. Finally, the teaching or suggestion to make the claimed invention must be found in the prior art and not in the applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). In addition, an obviousness rejection cannot be established in the first place unless each claim element is disclosed in the cited art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). The amended claims require incorporating at least two of the same sequence identifiers into a nucleic acid template. The amended claims are not obvious in view of the combination of the cited references because none of the cited references disclose or suggest that element of the amended claims.

Kawashima is limited to reporting different sequencing methods. Kawashima only reports annealing templates to primers and optionally then re-sequencing those templates with a second primer (Kawashima, paragraphs [0016] to [0108] and FIGs. 1A, 1B, 3A, 6A-6B, 9A-9B, 10A-10B, 12A-12C, 13A-13B, 14A-14B, and 16A-16B). Kawashima's methods do not analyze sequence reads to determine if identifiers remain associated with a template after a sequencing reaction for the purpose of avoiding false positive and/or false negative results. Importantly, Kawashima fails to disclose or suggest the element of the claims of incorporating at least two of the same sequence identifiers into a nucleic acid template. In fact, Kawashima does not contemplate that molecular overlap or mismatching can occur during a sequencing reaction.

The Office Action alleges that paragraph [00183] and Figs. 15d and 16b of Kawashima report incorporating at least two of the same sequence identifiers into a nucleic acid template. Applicants respectfully disagree.

While those sections of Kawashima report attaching "tag" sequences to a nucleic acid template, they do not disclose or suggest incorporating at least two of the same sequence identifiers into a nucleic acid template. Paragraph [0183] states:

> The procedure is the same as that described in the previous section except that in this case, the oligonucleotide linkers used to tail the randomly cut genomic DNA fragments are now made of two parts; the sequence of the primers grafted onto the surface (P1 and P2, FIG. 13b) and a "tag" sequence which is different for each

> sample and which will be used for identifying the origin of the DNA colony. Note that for each sample, the tag may not be unique, but a plurality of tags could be used. Hereafter, we will designate the DNA fragments obtained after such a suitable process by the expression: "tagged genomic DNA" (III, FIG. 13b).

That paragraph is limited to reporting that more than one tag may be used per template. Using more than one tag sequence per template does not does not disclose or suggest incorporating at least two of the same sequence identifiers into a nucleic acid template.

Like paragraph [0183], Figs. 15d and 16b merely report more than one tag sequence being used per nucleic acid template. Those figures are silent as to whether the tag sequences are the same. Additionally, Fig. 15 is not described in the as-filed application, so there is no identificqtion of the tag sequences being used in Fig. 15d. Fg. 16 is described at paragraph [0300] and that paragraph is silent as to whether the tag sequences are the same.

Accordingly, the sections of Kawashima cited in the Office Action fails to disclose or suggest the element of the claims of incorporating at least two of the same sequence identifiers into a nucleic acid template.

Parameswaran is limited to reporting incorporation of different barcode sequences into a single template (Parameswaran, page 4, right column and Tables 1-2). Parameswaran states:

> The forward and reverse barcodes varied in dinucleotide positioning along the length of the barcode. Positions 3, 4 (nucleotide J) and 6, 7 (nucleotide L) were homopolymeric dinucleotides in the forward barcode, while positions 4, 5 (nucleotide K) and 7, 8 (nucleotide M) were homopolymeric dinucleotides in the reverse barcode. Page 4, right column.

Accordingly, Parameswaran also fails to disclose or suggest the element of the claims of incorporating at least two of the same sequence identifiers into a nucleic acid template.

Kircher is a review article limiting to reporting different types of sequencing platforms. Like Kawashima, Kircher fails to disclose or suggest any methods for analyzing sequence reads to determine if identifiers remain associated with a template after a sequencing reaction for the purpose of avoiding false positive and/or false negative results. In fact, Kircher fails to disclose

or suggest any type of identifier, let alone a method that involves incorporating at least two of the same sequence identifiers into a nucleic acid template.

Accordingly, the amended claims are not obvious in view the combination of Kawashima, Parameswaran, and Kircher, because that combination of references fails to disclose or suggest the element of the amended claims of incorporating at least two of the same sequence identifiers into a nucleic acid template.

Applicants respectfully request withdrawal of the 35 U.S.C. §103(a) rejection.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: June 9, 2014

Respectfully submitted,
BROWN RUDNICK LLP

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

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(**Not for submission under 37 CFR 1.99**)

Application Number	13081660
Filing Date	2011-04-07
First Named Inventor	Gregory Porreca
Art Unit	1637
Examiner Name	T. E. Strzelecka
Attorney Docket Number	GSGE-001/01US 29258/14

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	Attorney Docket Number	GSGE-001/01US 29258/14

	1	Turner, et al., 2009, Methods for Genomic Partitioning, Annual Review of Genomics and Human Genetics 10:263-84.	☐
	2	Wallace, et al., 1979, Hybridization of synthetic oligodeoxyribonucteotides to dp x 174DNA:the effect of single base pair mismatch, Nucleic Acids Research 6:3543-3557.	☐
	3	Warner, et al., 1996, A general method for the detection of large CAG repeat expansions by fluorescent PCR, Journal Medical Genetics 33(12):1022-6.	☐
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	12	Yoshida, et al., 2004, Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage, Cancer Science 95(11)866-71.	☐
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	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2014-06-09
Name/Print	Thomas C. Meyers	Registration Number	36989

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Application Number:	13081660
Filing Date:	07-Apr-2011
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Filer:	Thomas C. Meyers/Michelle Aiello
Attorney Docket Number:	GSGE-001/01US 29258/14
Filed as Small Entity	

Utility under 35 USC 111(a) Filing Fees

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Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for Continued Examination	2801	1	600	600
		Total in USD ($)		**600**

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EFS ID:	19247240
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
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Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	09-JUN-2014
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	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

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	7	International Search Report and Written Opinion for WO2010/126614	☐
	8	International Search Report and Written Opinion mailed August 12, 2013, for International Patent Application No. PCT/US13/36575, filed April 15, 2013 (9 pages)	☐
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International application No. **PCT/US2013/032885**	International filing date *(day/month/year)* **19 March 2013 (19.03.2013)**	(Earliest) Priority Date *(day/month/year)* 09 April 2012 (09.04.2012)

Applicant

GOOD START GENETICS, INC.

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

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G06F 19/28(2011.01)i

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011-0034342 A1 (RICHARD FOX) 10 February 2011 See paragraphs [0015], [0035], [0040], [0119], [0165], [0173], [0178]; and fig. 1.	1,3,11,13
A		2,4-10,12,14-40
Y	US 2002-0187496 A1 (LEIF ANDERSSON et al.) 12 December 2002 See paragraphs [0003]-[0004], [0006], [0023], [0030], [0032], [0041], [0047], [0052], [0060], [0062], [0075], [0078], [0080]; and figs. 1-2, 4-6, 10-11.	1,3,11,13
A	EP 2437191 A2 (GENE SECURITY NETWORK LLC) 04 April 2012 See paragraphs [0002], [0024]-[0050]; and fig. 19.	1-40
A	WO 2002-093453 A2 (X-MINE, INC.) 21 November 2002 See paragraphs [0002], [0024]-[0027]; and figs. 3A-3B.	1-40
A	WO 2010-024894 A1 (23ANDME, INC.) 04 March 2010 See paragraph [0002]; and fig. 1.	1-40

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PCT/US2013/032885

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011-0034342 A1	10.02.2011	EP 2250594 A1	17.11.2010
		WO 2009-102901 A1	20.08.2009
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		WO 2009-3453 A2	21.11.2002
		WO 2009-3453 A3	11.03.2004
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		US 2010-0057807 A1	04.03.2010
		US 8428886 B2	23.04.2013

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
TOSTI ROBERT J.

BROWN RUDNICK LLP ONE FINANCIAL CENTER
BOSTON MA 02111 USA

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing *(day/month/year)*	**28 June 2013 (28.06.2013)**
Applicant's or agent's file reference GSGE-010/01WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2013/032885	**19 March 2013 (19.03.2013)**	09 April 2012 (09.04.2012)

International Patent Classification (IPC) or both national classification and IPC

G06F 19/28(2011.01)i

Applicant

GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.
For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/KR	Date of completion of this opinion	Authorized officer
Korean Intellectual Property Office 189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea Facsimile No. 82-42-472-7140	25 June 2013 (25.06.2013)	BYUN, Sung Cheal Telephone No. 82-42-481-8262

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/032885

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of :

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

   a. a sequence listing filed or furnished
      ☐ on paper
      ☐ in electronic form

   b. time of filing or furnishing
      ☐ contained in the international application as filed.
      ☐ filed together with the international application in electronic form.
      ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additioanl copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/032885

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-40	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	2,4-10,12,14-40	YES
	Claims	1,3,11,13	NO
Industrial applicability (IA)	Claims	1-40	YES
	Claims	NONE	NO

2. Citations and explanations :

Reference is made to the following documents:

D1: US 2011-0034342 A1 (RICHARD FOX) 10 February 2011
D2: US 2002-0187496 A1 (LEIF ANDERSSON et al.) 12 December 2002
D3: EP 2437191 A2 (GENE SECURITY NETWORK LLC) 04 April 2012
D4: WO 2002-093453 A2 (X-MINE, INC.) 21 November 2002
D5: WO 2010-024894 A1 (23ANDME, INC.) 04 March 2010

2.1 Novelty and Inventive Step

2.1.1 Independent Claim 1 and Dependent Claims 2-10

D1, which is considered to be the closest prior art to the subject matter of claim 1, discloses a method comprising:

retrieving from one or more computer-readable storage devices a first variant object comprising a description of a first mutation as a first variant of a reference and a second variant object comprising a description of a second mutation as a second variant of the reference, each of the first and second variant objects being an instance of an abstract class of a genomic feature (retrieving from a library each variant which describe properties of molecular variants, see paragraphs [0119], [0165] and fig. 1 in D1);

determining a relationship between the first and second mutations (determining mutations and establishing relationships between the mutations, see paragraphs [0050], [0118] and fig. 1 in D1);

retrieving a third object comprising a description of the relationship, the third object also being an instance of the abstract class (receiving a data set of mutations which established relationships between the various members of the data, see paragraphs [0050], [0165] and fig. 1

Continued on Supplemental Box

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2013/032885

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

In claim 4, the term "the memory" has not been previously defined. Therefore, claim 4 is not clear, contrary to the requirement of PCT Article 6.

In claims 8 and 18, the term "the information" has not been previously defined. Therefore, claims 8 and 18 are not clear, contrary to the requirement of PCT Article 6.

In claim 24, the term "the one or more computer-readable storage devices" has not been previously defined. Therefore, claim 24 is not clear, contrary to the requirement of PCT Article 6.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box No. V

in D1); and

providing a description of the variant based on the third object (describing properties of the molecular variants of objective data, see paragraph [0119] and fig. 1 in D1).

The subject matter of claim 1 differs from that of D1 in that claim 1 comprises the feature of receiving genetic data relating to an individual, the data representing at least a first mutation and a second mutation. However, this feature can readily be predicted from D2 that discloses the feature of obtaining genetic information about sequence variants from the sampled individuals' information (see paragraphs [0006], [0030], [0041] and figs. 1-2, 4 in D2). Accordingly, claim 1 would have been obvious over D1 in view of D2. Therefore, claim 1 lacks an inventive step under PCT Article 33(3).

The additional feature (the one or more computer-readable storage devices comprise a relational database) of claim 3 dependent on claim 1 can readily be predicted from D1 that discloses the feature of one computer comprising a database capable of storing relationships between the data (see paragraphs [0050], [0199] and fig. 1 in D1). Accordingly, claim 3 would have been obvious over D1 in view of D2. Therefore, claim 3 lacks an inventive step under PCT Article 33(3).

The additional features of claims 2 and 4-10 directly or indirectly dependent on claim 1 are not disclosed in any of the prior art documents, nor are they obvious to a person skilled in the art over the documents. Accordingly, claims 2 and 4-10 are novel and involve an inventive step under PCT Article 33(2) and (3).

2.1.2 Independent Claim 11 and Dependent Claims 12-20

The features of independent claim 11 essentially correspond to those of claim 1, except for the category of the invention and an added feature of a processor (a processor, see paragraph [0078] and fig. 11 in D2). Therefore, for the same reasons as those of claim 1, claim 11 lacks an inventive step under PCT Article 33(3).

The additional feature (the database is a relational database) of claim 13 dependent on claim 11, can readily be predicted from D2 that discloses the feature of a relationship between database objects (see paragraph [0023] and fig. 2 in D2). Accordingly, claim 13 would have been obvious over D1 in view of D2. Therefore, claim 13 lacks an inventive step under PCT

Continued on The Next Page

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Previous Page

Article 33(3).

The additional features of claims 12 and 14-20 directly or indirectly dependent on claim 11 are not disclosed in any of the prior art documents, nor are they obvious to a person skilled in the art over the documents. Accordingly, claims 12 and 14-20 are novel and involve an inventive step under PCT Article 33(2) and (3).

2.1.3 Independent Claim 21 and Dependent Claims 22-30

The subject matter of claim 21 differs from these prior art documents in that a method in claim 21 comprises: providing, using a computer comprising a memory coupled to a processor, code describing an abstract class of genomic feature object and creating relation objects, each relation object being an instance of the genomic feature object and comprising one or more genomic feature objects and description of a relationship among the one or more genomic feature objects. And it is not obvious to a person skilled in the art over the documents taken alone or in combination. Therefore, claim 21 meets the requirements of PCT Article 33(2) and (3) with respect to novelty and inventive step.

Claims 22-30 are dependent on claim 21 and therefore meet the requirements of PCT Article 33(2) and (3).

2.1.4 Independent Claim 31 and Dependent Claims 32-40

The features of independent claim 31 essentially correspond to those of claim 21, except for the category of the invention and an added feature of a processor (see paragraph [0078] and fig. 11 in D2). Therefore, for the same reasons as those of claim 21, claim 31 meets the requirements of PCT Article 33(2) and (3) with respect to novelty and inventive step.

Claims 32-40 are dependent on claim 31 and therefore meet the requirements of PCT Article 33(2) and (3).

2.2 Industrial Applicability

Claims 1-40 are industrially applicable under PCT Article 33(4).

Electronic Acknowledgement Receipt	
EFS ID:	19246844
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	09-JUN-2014
Filing Date:	07-APR-2011
Time Stamp:	17:09:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	SchadtHurmMolecularGen2010.PDF	1623865 62396b83a614553549c2e86272739b9c171c894b	no	14
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Information:					

2	Non Patent Literature	Schatz-GenomeResearch.PDF	971875 bdfde8178ef821b65825cc2924ffba1e22d1a7d91	no	9
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3	Non Patent Literature	SchrijverJMolDiag2005.PDF	1126745 3c58e4dcbdc8ef7631c6551c4ca3abf9d88b6f84	no	11
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4	Non Patent Literature	Schuette1995JPBA.PDF	1928851 9c08cf35ec4009b8e73b30185282b647344874d7	no	9
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5	Non Patent Literature	Schwartz-2009-JMolDiag.PDF	279927 4e9da494d964152870db82e7dc9de9bf30882956	no	5
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8	Non Patent Literature	--Sievers-et-al_Molecular_systems_biology_2011.PDF	227187 6b764ab0b8d655e4ef5c334972fa74d36f3f3890	no	6
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9	Non Patent Literature	Simpson-2009-GenomeResearch.PDF	785522 a2b1b9b4e00326f3737614ff4619529e9562f700	no	7
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39	Non Patent Literature	ISR_and_Written_Opinion_for_PCTUS2013032885.PDF	358391 e2593744446a32dff87bc7f90af68b8b7a5ff4a7	no	9
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40	Non Patent Literature	KurtzGenomeBiology2004.PDF	8293048 7c102369a7004be70c29f96e670a4a4d7ad4f251	no	9
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41	Non Patent Literature	Quail-ELS-2010.PDF	1912404 bfc5975695ee805e267046e51b8547def852256c	no	5
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42	Non Patent Literature	Sauro-How-Do-You-Calculate-2004.PDF	142346 00d24abbda607cafbde88c8c684a9713180199ef	no	3
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43	Non Patent Literature	Sauro-Z-Score-2004.PDF	127509 223b3c482a3da2384a02e4c63d5dd9718994f035	no	2
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44	Non Patent Literature	Tokino-Hum-Genet-1996.PDF	639093 beb698f66ccf47448026c8400d4449eab93a2820	no	7
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45	Non Patent Literature	TurnerNatureMeth2009OriginalandSupplement.PDF	1232618 83e4fba6f9a9ab15819a4035ad389d16f1b03484	no	14
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46	Non Patent Literature	Wu1998AnalBiochem.PDF	4725990 ee3180d5663dbdbb9e2f6e5ac21c8a519e4ada07	no	10
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47	Foreign Reference	EP1321477A1.PDF	5877566 65fc785a8a22f8c2cac9169f13480d5b20fdd268	no	113
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48	Foreign Reference	EP1564306A2.PDF	1749054 fe4f7c49e73ad66d187daa79727e72ed94be2f19	no	33
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49	Foreign Reference	EP2437191A2.PDF	4716062 8aa945e790401cb0c3cc6fd8f336999fb8e0a6e0	no	73
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51	Foreign Reference	WO2004018497.PDF	4622852 04a98da47baeb95327ee521d9b4442d080bb0521	no	134
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52	Foreign Reference	WO2007010251.PDF	4124692 93f2418a76055e1f0e2ec0a805a51a15586ed0f2	no	99
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53	Foreign Reference	WO2007123744.PDF	5483019 91232c26b44eafe0d997263557fc62accf19c49b	no	135
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57	Foreign Reference	WO2007107717.PDF	1917888 8667970a0f9f9be52f15c3794039cf0780e0dcda	no	43
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58	Non Patent Literature	Soni-2007-Clinical.pdf	700252 e6f66956898f9141cccbc70962ccaa268d1eadcc	no	6
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			Total Files Size (in bytes):	117785017	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/081,660	Filing Date 04/07/2011	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **06/09/2014**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 40	Minus	** 46	= 0	X $40 =	0
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $210 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/KIM DOWNING/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT **(Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					
If you wish to add additional U.S. Patent citation information please click the Add button.						

U.S.PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					
If you wish to add additional U.S. Published Application citation information please click the Add button.						

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐
If you wish to add additional Foreign Patent Document citation information please click the Add button								

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

	1	Smirnov, et al., 1996, Sequencing oligonucleotides by exonuclease digestion and delayed extraction matrix-assisted laser desorption ionization time-of-flight mass spectrometry, Anal Biochem 238(1):19–25.	☐
	2	Alazard, et al., 2005, Sequencing Oligonucleotides by Enrichment of Coupling Failures Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry, Current Protocols in Nucleic Acid Chemistry 10.10.1-10.10.7.	☐
	3	Kumar, S., et al., 2010, Comparing de novo assemblers for 454 transcriptome data, Genomics 11:571.	☐
	4		☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2014-07-01
Name/Print	Thomas C. Meyers	Registration Number	36989

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	19448721
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	01-JUL-2014
Filing Date:	07-APR-2011
Time Stamp:	13:33:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Smirnov-1996-AnalBioch.PDF	845402 dd2d816d51456a794336417d362a0dc9c03e8caf	no	7
Warnings:					
Information:					

2	Non Patent Literature	Alazard2005CPNAC.PDF	413930 ae947d0e0d36acdbbc5f975edf65b4515688b0e6	no	7
Warnings:					
Information:					
3	Non Patent Literature	Kumar-2010-BMCGenomics.PDF	6195666 b67c0ecf1c7dc7c3e04001c7764e72da0bdb1277	no	12
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	GSGE-001-01US-SupplementalIDS.pdf	190405 0a73b43a774b3e0b76c97238082d42c9b401ea45	no	4
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
		Total Files Size (in bytes):	7645403		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/80 (07-12)
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POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number: 21710

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☒ The address associated with Customer Number: 21710

OR

☐	Firm or Individual Name			
	Address			
	City		State	Zip
	Country			
	Telephone		Email	

Assignee Name and Address: Good Start Genetics, Inc.
237 Putnam Avenue
Cambridge, MA 02139

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature	Gregory J. Porreca	Date	12-4-2013
Name	Gregory J. Porreca	Telephone	617-714-0855
Title	Vice President of Research and Techology		

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Gregory Porreca et al.

Application No./Patent No.: 13/081,660 Filed/Issue Date: April 7, 2011

Titled: METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

Good Start Genetics, Inc. , a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. [X] The assignee of the entire right, title, and interest.

2. [ ] An assignee of less than the entire right, title, and interest (check applicable box):

   [ ] (The extent (by percentage) of its ownership interest is _____ %). Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   [ ] There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. [ ] The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. [ ] The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2, or 3 above (not option 4) is evidence by either (choose **one** of options A or B below):

A. [X] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 027200 , Frame 0369 , or for which a copy thereof is attached.

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: _____ To: _____
      The document was recorded in the United States Patent and Trademark Office at
      Reel _____ , Frame _____ , or for which a copy thereof is attached.

   2. From: _____ To: _____
      The document was recorded in the United States Patent and Trademark Office at
      Reel _____ , Frame _____ , or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

4. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Thomas C. Meyers/	August 13, 2014
Signature	Date
Thomas C. Meyers	Attorney for Assignee
Printed or Typed Name	Title or Registration Number

Electronic Acknowledgement Receipt	
EFS ID:	19851270
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	13-AUG-2014
Filing Date:	07-APR-2011
Time Stamp:	13:05:07
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	General_POA_Good_Start_Genetics.PDF	104443 a7530b6564dcedbddfd8755401f74a0a27641121	no	1
Warnings:					
Information:					

2	Assignee showing of ownership per 37 CFR 3.73.	373C.PDF	24603 27b173017f28e27335a9549c0b0fe4f1bbb50215	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	129046

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14

CONFIRMATION NO. 4441

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

POA ACCEPTANCE LETTER

OC000000070260568

Date Mailed: 08/20/2014

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/13/2014.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/qtran/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

21710 7590 09/11/2014
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

NOTIFICATION DATE	DELIVERY MODE
09/11/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

Office Action Summary	Application No. 13/081,660	Applicant(s) PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *6/9/14*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-4,7-32,35-38 and 41-46* is/are pending in the application.

5a) Of the above claim(s) *4,9,17-19,23,26,37 and 42-46* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-3,7,8,10-16,20-22,24,25,27-32,35,36,38 and 41* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *6/9/14; 7/1/14*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

1. The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on June 9, 2014 has been entered.

3. Claims 1-46 were previously pending, with claims 4, 6, 9, 17-19, 23, 26, 33, 34, 37, 40 and 42-46 withdrawn from consideration. Applicant amended claims 1, 20 and 35, and cancelled claims 5, 6, 33, 34, 39 and 40. Claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38 and 41 will be examined.

4. Applicant's amendments did not overcome any of the previously presented rejections for reasons given in the "Response to Arguments" below.

Information Disclosure Statement

5. The information disclosure statement (IDS) submitted on June 9, 2014 was filed after the mailing date of the final office action on March 7, 2014. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

6. The information disclosure statement (IDS) submitted on July 1, 2014 was filed after the mailing date of the final office action on March 7, 2014. The submission is in compliance with

the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Response to Arguments

7. Applicant's arguments filed June 9, 2014 have been fully considered but they are not persuasive.

A) Regarding the rejection of claims 1-3, 7, 8, 10-12, 16, 20-22, 24-25, 27-29, 35, 36 and 38 under 35 U.S.C. 102(b) over Parameswaran et al. Applicant argues that the amendments to claims 1, 20 and 35 incorporate limitations of claims which were not previously rejected as anticipated by this reference. However, upon review of the description of the term "barcode" in the specification, the Parameswaran et al. reference still anticipates the amended claims.

The rejection is maintained.

B) Regarding the rejection of claims 1-3, 5, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, 39 and 41 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kawashima et al., Parameswaran et al. and Kircher et al., Applicant argues the following:

"Kawashima is limited to reporting different sequencing methods. Kawashima only reports annealing templates to primers and optionally then re-sequencing those templates with a second primer (Kawashima, paragraphs [0016] to [0108] and FIGs. 1A, 1B, 3A, 6A-6B, 9A-9B, 10A-10B, 12A-12C, 13A-13B, 14A-14B, and 16A-16B). Kawashima's methods do not analyze sequence reads to determine if identifiers remain associated with a template after a sequencing reaction for the purpose of avoiding false positive and/or false negative results. Importantly, Kawashima fails to disclose or suggest the element of the claims of incorporating at least two of

the same sequence identifiers into a nucleic acid template. In fact, Kawashima does not contemplate that molecular overlap or mismatching can occur during a sequencing reaction.

The Office Action alleges that paragraph [00183] and Figs. 15d and 16b of Kawashima report incorporating at least two of the same sequence identifiers into a nucleic acid template. Applicants respectfully disagree.

While those sections of Kawashima report attaching "tag" sequences to a nucleic acid template, they do not disclose or suggest incorporating at least two of the same sequence identifiers into a nucleic acid template. Paragraph [0183] states:

> The procedure is the same as that described in the previous section except that in this case, the oligonucleotide linkers used to tail the randomly cut genomic DNA fragments are now made of two parts; the sequence of the primers grafted onto the surface (P1 and P2, FIG. 13b) and a "tag" sequence which is different for each sample and which will be used for identifying the origin of the DNA colony. Note that for each sample, the tag may not be unique, but a plurality of tags could be used. Hereafter, we will designate the DNA fragments obtained after such a suitable process by the expression: "tagged genomic DNA" (III, FIG. 13b).

That paragraph is limited to reporting that more than one tag may be used per template. Using more than one tag sequence per template does not does not disclose or suggest incorporating at least two of the same sequence identifiers into a nucleic acid template.

Like paragraph [0183], Figs. 15d and 16b merely report more than one tag sequence being used per nucleic acid template. Those figures are silent as to whether the tag sequences are the same. Additionally, Fig. 15 is not described in the as-filed application, so there is no

identification of the tag sequences being used in Fig. 15d. Fg. 16 is described at paragraph [0300] and that paragraph is silent as to whether the tag sequences are the same.

Accordingly, the sections of Kawashima cited in the Office Action fails to disclose or suggest the element of the claims of incorporating at least two of the same sequence identifiers into a nucleic acid template.

Parameswaran is limited to reporting incorporation of different barcode sequences into a single template (Parameswaran, page 4, right column and Tables 1-2). Parameswaran states:

> The forward and reverse barcodes varied in dinucleotide positioning along the length of the barcode. Positions 3, 4 (nucleotide J) and 6, 7 (nucleotide L) were homopolymeric dinucleotides in the forward barcode, while positions 4, 5 (nucleotide K) and 7, 8 (nucleotide M) were homopolymeric dinucleotides in the reverse barcode. Page 4, right column.

Accordingly, Parameswaran also fails to disclose or suggest the element of the claims of incorporating at least two of the same sequence identifiers into a nucleic acid template.

Kircher is a review article limiting to reporting different types of sequencing platforms. Like Kawashima, Kircher fails to disclose or suggest any methods for analyzing sequence reads to determine if identifiers remain associated with a template after a sequencing reaction for the purpose of avoiding false positive and/or false negative results. In fact, Kircher fails to disclose or suggest any type of identifier, let alone a method that involves incorporating at least two of the same sequence identifiers into a nucleic acid template.

Accordingly, the amended claims are not obvious in view the combination of Kawashima, Parameswaran, and Kircher, because that combination of references fails to disclose or suggest the element of the amended claims of incorporating at least two of the same sequence identifiers into a nucleic acid template."

However, Kawashima teaches that different tags are used for different samples, not for different templates, i.e., templates from the same sample would have the same tags, in view of the drawings, which clearly show "tag" on both sides of a target sequence, not "tag1" and "tag2". Further, the fact that Kawashima states that templates within the same sample may have different tags does not mean that two different tags are present within a single template, but that two templates have tags that differ from each other.

The rejection is maintained.

Claim Interpretation

8. Applicant did not define the term "sequence identifier" or "barcode", therefore any sequence of two or more nucleotides is interpreted as a "sequence identifier" or a "barcode".

Claim Rejections - 35 USC § 102

9. The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

10. Claims 1-3, 7, 8, 10-12, 16, 20-22, 24, 25, 27-29, 35, 36 and 38 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Parameswaran et al. (Nucl. Acids Res., vol. 35, e130, pp. 1-9, 2007; previously cited).

Claims 1, 2, 10, 16, 20, 21, 27 and 35 are considered together in claim 35, since it contains limitations of all of these claims.

Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Parameswaran et al. teach a method comprising:

obtaining a nucleic acid template (page 2, third paragraph);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template, wherein the first and second barcode sequences are the same (page 2, paragraphs 4-6; page 3; page 4, first and second paragraphs; Fig. 1. As can be seen from the supplementary Table 1A, for sample 41, for example, the sequences AGC and GCT in the barcode part of the primer are the same in the forward and reverse primers. Therefore Parameswaran et al. teach barcodes with the same sequences.);

amplifying the template (page 4, third paragraph);

sequencing the template to generate sequence reads (page 4, third paragraph), and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with a template (page 7, paragraphs 2-4; page 8, first and second paragraph; Table 3).

Regarding claims 3, 22 and 36, Parameswaran et al. teach the template having the following configuration: 5'-F-adapter (=first known sequence)- F-barcode (=a first barcode sequence)-F-cloning linker (=a second known sequence)-target sequence-RC (R-cloning linker)

(=a third known sequence)-RC (R-barcode) (= a second barcode sequence)-RC(R-adapter) (= a fourth known sequence) (Fig. 1).

Regarding claims 7, 8, 24, 25 and 38, Parameswaran et al. teach the template undergoing emulsion PCR, therefore they inherently teach template directly immobilized on a solid support (page 4, third paragraph).

Regarding claims 11, 12, 28 and 29, Parameswaran et al. teach pyrosequencing (page 4, third paragraph), therefore they inherently teach single molecule sequencing by synthesis, since each DNA molecule is sequenced individually.

Claim Rejections - 35 USC § 103

11. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38 and 41 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kawashima et al. (US 2005/0100900 A1; published May 2005; previously cited), Parameswaran et al. (Nucl. Acids Res., vol. 35, e130, pp. 1-9, 2007; previously cited) and Kircher et al. (Bioessays, vol. 32, pp. 524-536, June 2010; previously cited).

A) Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Kawashima et al. teach a method comprising:

obtaining a nucleic acid template (page 4, [0049]-[0051]; [0056]);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template, wherein the first and second barcode sequences are the same (page 2, [0026]-[0029]; page 4, [0052]-[0053]; Fig. 15, page 13, [0182]-[0189], where the tag sequences are the barcodes, and the tags are the same as indicated in Fig. 15d, Fig. 16b and where the tag identifies a sample, but not the nucleic acid strand);

amplifying the template (Fig. 1; page 6, [0109]; page 7, [0110]-[0124]; page 10, [0149]-[0157]); and

sequencing the template (Fig. 17; page 15-18).

Regarding claims 3, 22 and 36, Kawashima et al. teach the template having the following configuration: 5'-P1 primer (=first known sequence)- tag (=a first barcode sequence)-S1 (=a second known sequence)-target sequence-S2 (=a third known sequence)-tag (= a second barcode sequence)-P2 primer (= a fourth known sequence) (Fig. 15d, Fig. 16b).

Regarding claims 7, 8, 24, 25 and 38, Kawashima et al. teach the template directly immobilized on a solid support (Fig. 1).

Regarding claims 11, 12, 28 and 29, Kawashima et al. teach single molecule sequencing by synthesis (Fig. 17, page 5, [0067]-[0075]; page 15, [0233]-[0238]).

Regarding claims 13, 30 and 41, Kawashima et al. teach hybridizing a primer to the template, contacting the duplex with a polymerase in the presence of detectably labeled nucleotide, detecting the signal from incorporated nucleotide and repeating the process to determine the sequence of nucleic acid (Fig. 17; page 15, [0238]-[0245]).

Regarding claims 14, 15, 31 and 32, Kawashima et al. teach fluorescently labeled nucleotides (page 15, [0239]-[0241]).

B) Kawashima et al. do not teach that the barcodes can be used to validate the integrity of nucleic acid templates.

C) Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Parameswaran et al. teach a method comprising:

obtaining a nucleic acid template (page 2, third paragraph);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (page 2, paragraphs 4-6; page 3; page 4, first and second paragraphs; Fig. 1);

amplifying the template (page 4, third paragraph);

sequencing the template to generate sequence reads (page 4, third paragraph), and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with a template (page 7, paragraphs 2-4; page 8, first and second paragraph; Table 3).

Regarding claims 3, 22 and 36, Parameswaran et al. teach the template having the following configuration: 5'-F-adapter (=first known sequence)- F-barcode (=a first barcode sequence)-F-cloning linker (=a second known sequence)-target sequence-RC (R-cloning linker) (=a third known sequence)-RC (R-barcode) (= a second barcode sequence)-RC(R-adapter) (= a fourth known sequence) (Fig. 1).

Regarding claims 7, 8, 24, 25 and 38, Parameswaran et al. teach the template undergoing emulsion PCR, therefore they inherently teach template directly immobilized on a solid support (page 4, third paragraph).

Regarding claims 11, 12, 28 and 29, Parameswaran et al. teach pyrosequencing (page 4, third paragraph), therefore they inherently teach single molecule sequencing by synthesis, since each DNA molecule is sequenced individually.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the barcodes of Kawashima et al. to validate the integrity of the nucleic acid molecules being sequenced, as suggested by Parameswaran et al. (page 8, last paragraph):

"Our barcode specification has been tailored for use with pyrosequencing technologies, particularly reflecting restrictions imposed on the generation of homopolymeric runs. These barcodes may also be compatible with other sequencing platforms. Optimally, however the precise structure of the barcodes might need to be revised to reflect sequencing error profiles of the various systems. If necessary, multiple sequencing primers may also be used to identify the barcode and the sequence of interest, to overcome limitations imposed by short read lengths. As the availability of high-throughput technology advances, massively multiplexed barcoded sequencing will be extremely useful in performing surveys of diverse systems for nucleic acid signatures."

As stated by Kircher et al. regarding the type of sequencing performed by the method of Kawashima et al. (page 527, last three paragraphs; page 528; Fig. 3):

"The Illumina library and flow cell preparation includes several in vitro amplification steps, which cause a high background error rate and contribute to the average error rate of about 10^{-2}–10^{-3} [41, 42]. Further, the flow cell preparation creates a fraction of ordinary-looking clusters that are initiated from more than one individual sequence. These results in mixed signals

and mostly low quality sequences for these clusters. Similar to the 454 ghost wells, the Illumina image analysis may identify chemistry crystals, dust, and lint particles as clusters and call sequences from these. In such cases the resulting sequences typically appear to be of low sequence complexity."

Therefore one of ordinary skill in the art at the time of the invention would have been motivated to use error detection using barcode sequences to verify correctness of sequencing results as suggested by Parameswaran et al. in the method of Kawashima et al. to avoid sequencing errors resulting from sample preparation and processing described by Kircher et al.

13. No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TERESA E STRZELECKA
Primary Examiner
Art Unit 1637

/TERESA E STRZELECKA/
Primary Examiner, Art Unit 1637
September 3, 2014

Notice of References Cited	Application/Control No. 13/081,660	Applicant(s)/Patent Under Reexamination PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Parameswaran, P. et al., Nucl. Acids Res., vol. 35, e130, Supplementary Material (2007)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /TS/

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	Attorney Docket Number	GSGE-001/01US 29258/14

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	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

EXAMINER SIGNATURE			
Examiner Signature	/Teresa Strzelecka/	Date Considered	09/02/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

WEST Search History for Application 13081660

Creation Date: 2014090217:28

Prior Art Searches

Query	DB	Op.	Plur.	Thes.	Date
6403320.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
20090163366	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20080269068	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20100035243 or 20100297626 or 20100330619	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20070166705	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20090226975	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20050100900	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
7115400.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
7057026.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parrallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6 or barcod$5) and sequencing	USPT	ADJ	YES	ASSIGNEE	06-10-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6 or barcod$5) and sequencing	USPT	ADJ	YES	ASSIGNEE	06-10-2013
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(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical)	USPT	ADJ	YES	ASSIGNEE	09-02-2014
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((barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical)) same sequencing	USPT	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical) same librar$5	USPT	ADJ	YES	ASSIGNEE	09-02-2014
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multiple near3 (barcod$5 or index$5) same sequencing	DWPI	ADJ	YES	ASSIGNEE	09-02-2014
US-20080269068-A1.did.	PGPB	ADJ	YES	ASSIGNEE	09-02-2014

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	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

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=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

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	46	N	N	N						

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	4		☐

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	32	Li H. & Durbin R., 2010, (2010) Fast and accurate long-read alignment with Burrows-Wheeler Transform. Bioinformatics, Epub.	☐
	33	Li, et al., 2008, SOAP: short oligonucleotide alignment program, Bioinformatics 24(5):713-14.	☐

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Day : Tuesday
Date: 9/2/2014
Time: 09:43:58

Inventor Name Search

Enter the **first few letters** of the Inventor's Last Name.
Additionally, enter the **first few letters** of the Inventor's First name.

Last Name	**First Name**	
Porreca	Gregory	Search

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Last Name	**First Name**	
Church	George	Search

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PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /TS/

U.S.PATENTS						Remove
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	4683195		1987-07-28	Mullis et al.	
	2	4683202		1987-07-28	Mullis	
	3	4988617		1991-01-29	Landegren et al.	
	4	5234809		1993-08-10	Akzo N.V.	
	5	5242794		1993-09-07	Whiteley et al.	
	6	5494810		1996-02-27	Barany et al.	
	7	5583024		1996-12-10	McElroy, deceased et al.	
	8	5604097		1997-02-18	Brenner	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /TS/

	9	5636400		1997-06-10	Young	
	10	5674713		1997-10-07	McElroy, deceased et al.	
	11	5695934		1997-12-09	Brenner	
	12	5700673		1997-12-23	McElroy, deceased et al.	
	13	5701256		1997-12-23	Marr et al.	
	14	5830064		1998-11-03	Bradish et al.	
	15	5846719		1998-12-08	Brenner et al.	
	16	5863722		1999-01-26	Brenner	
	17	5866337		1999-02-02	Schon	
	18	5869252		1999-02-09	Bouma et al.	
	19	5871921		1999-02-16	Landegren et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /TS/

	20	5993611		1999-11-30	Moroney, III et al.	
	21	6100099		2000-08-08	Gordon et al.	
	22	6138077		2000-10-24	Brenner	
	23	6150516		2000-11-21	Brenner et al.	
	24	6172214		2001-01-09	Brenner	
	25	6172218		2001-01-09	Brenner	
	26	6197508		2001-03-06	Stanley	
	27	6210891		2001-04-03	Nyren et al.	
	28	6223128		2001-04-24	Allex et al.	
	29	6235472		2001-05-22	Landegren et al.	
	30	6235475		2001-05-22	Brenner et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /TS/

	31	6235501		2001-05-22	Gautsch et al.	
	32	6258568		2001-07-10	Nyren	
	33	6274320		2001-08-14	Rothberg et al.	
	34	6306597		2001-10-23	Macevicz	
	35	6352828		2002-03-05	Brenner	
	36	6361940		2002-03-26	Van Ness et al.	
	37	6489105		2002-12-03	Matlashewski et al.	
	38	6558928		2003-05-06	Landegren	
	39	6582938		2003-06-24	Su et al.	
	40	6585938		2003-07-01	Machida et al.	
	41	6714874		2004-03-30	Myers et al.	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
IDS (5/24/12; 8/6/12; 5/6/13)	6/7/13	/TS/
Inventor name search	6/7/13	/TS/
WEST	6/7/13, 6/10/13	/TS/
IDS (6/9/14, 7/1/14)	9/2/14	/TS/
WEST	9/2/14	/TS/
Inventor name search	9/2/14	/TS/

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT **(Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
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	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					
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U.S.PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	1996/019586	WO	A1	1996-06-27	Visible Genetics Inc		☐
	2	1998/014275	WO	A1	1998-04-09	Intronn Llc		☐
	3	2004/083819	WO	A2	2004-09-30	Trace Genetics, Inc		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

	4	2005/003304	WO	A2	2005-01-13	Illumina, Inc		☐

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	Thompson, et al., 2011, "The properties and applications of single-molecule DNA sequencing," Genome Biology 12(2):217, 10 pages.	☐
	2	Australian Patent Examination Report No. 1 issued August 12, 2014, for Australian Patent Application No. 2010242073 filed April 30, 2010, 4 pages.	☐
	3	International Search Report and Written Opinion mailed September 3, 2014, for International Patent Application No. PCT/US2014/027324, filed March 14, 2014, 7 pages.	☐
	4	Supplementary European Search Report issued August 26, 2014, for European Patent Application No. 12765217.0, filed March 20, 2012, 5 pages.	☐

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2014-10-15
Name/Print	Thomas C. Meyers	Registration Number	36989

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	20396709
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Steve Whitaker
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	15-OCT-2014
Filing Date:	07-APR-2011
Time Stamp:	09:05:23
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Thompson-2011-GenomeBiol.PDF	440819 e60f684cb72bf8344ebb6e22d2faa8615c42a6dd	no	10
Warnings:					
Information:					

2	Non Patent Literature	APER-for-AU2010242073.PDF	106866 b75b376bc996e39c7d6f895e7788803c25f5e3e9	no	4
Warnings:					
Information:					
3	Non Patent Literature	ISRWO-for-PCT-US2014-027324.PDF	446146 cbe4a4618f82a1c18b6146497ea9a55b700a3ca5	no	7
Warnings:					
Information:					
4	Non Patent Literature	SESR-for-EP12765217.PDF	413065 5e2b780661a8490ba7321a075c2857d0fa2e1005	no	5
Warnings:					
Information:					
5	Foreign Reference	WO1996019586.PDF	1044274 a16821bf77614a932b76010347f497430cef03fa	no	27
Warnings:					
Information:					
6	Foreign Reference	WO1998014275.PDF	1382425 54ed640163171c240d538335597b8a4b653449dc	no	27
Warnings:					
Information:					
7	Foreign Reference	WO2004083819A2.PDF	1574928 a7e691af2fa371c6c43b14393d5c4cb7ac841a93	no	34
Warnings:					
Information:					
8	Foreign Reference	WO2005003304.PDF	8811059 a978816da74d7dc1d7bd3c8af0cd0b45d3e08e81	no	156
Warnings:					
Information:					
9	Information Disclosure Statement (IDS) Form (SB08)	Information_Disclosure_Statement.pdf	1210107 941a331932dfba683fe470cd6b6444e3f74e209c	no	4
Warnings:					
Information:					
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		Total Files Size (in bytes):	15429689		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference GSGE-016/01WO 29258/76	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 14/27324	International filing date *(day/month/year)* 14 March 2014 (14.03.2014)	(Earliest) Priority Date *(day/month/year)* 14 March 2013 (14.03.2013)
Applicant GOOD START GENETICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. _____

   ☐ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT PCT/US2014/027324 03.09.2014

International application No.
PCT/US 14/27324

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, C12P 19/34 (2014.01)
USPC - 435/6.11, 435/91.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 435/6.11, 435/91.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- C12Q1/6883, C12Q1/6837, C12Q1/6827, C12Q1/686, C12Q2525/161, C12Q2537/143, C12Q2549/113, C12Q2561/125, C12Q1/6869; C12Q2521/501; C12Q2533/107; C12Q2535/122 (2014.01); USPC- 435/6.1, 435/6.12, 435/91.2, 506/2, 506/4

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: GOOD START GENETICS, INC.; UMBARGER, Mark; tiling, molecular inversion probes, MIP, adjacent probes, hybridize, opposite, antisense, strand, nucleic acid template, overlapping, filled-in region, Illumina, single molecule sequencing-by synthesis, universal portion, flanked

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2012/0165202 A1 (PORRECA, et al.) 28 June 2012 (28.06.2012) Abstract, claim 1; Figs 1, 11; para [0014], [0123], [0133], [0149], [0150], [0155], [0171], [0175], [0198], [0220]-[0224], [0235]-[0240], [0276], [0295], [0339]-[0354], [0376]-[0381]	1-3, 6-13 ---------- 4, 5, 14, 15
Y	THOMPSON, et al. The properties and applications of single-molecule DNA sequencing. Genome Biol. 2011, 12(2):217; Abstract, pg 3, col 2; pg 5, Fig 2 and its legend	4, 5
Y	US 2008/0269068 A1 (CHURCH, et al.) 30 October 2008 (30.10.2008) Abstract, claims 1, 6, 7; para [0081]-[0083]	14, 15

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2014 (28.07.2014)	03 SEP 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: THOMAS C. MEYERS
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	03 SEP 2014
Applicant's or agent's file reference GSGE-016/01WO 29258/76	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 14/27324	14 March 2014 (14.03.2014)	14 March 2013 (14.03.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68, C12P 19/34 (2014.01)
USPC - 435/6.11, 435/91.1

Applicant GOOD START GENETICS, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	28 July 2014 (28.07.2014)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 14/27324

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 14/27324

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-15	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-15	NO
Industrial applicability (IA)	Claims	1-15	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-3 and 6-13 lack an inventive step under PCT Article 33(3) as being obvious over US 2012/0165202 A1 to Porreca et al. (hereinafter "Porreca").

As to claim 1, Porreca discloses a method for analyzing a nucleic acid template, the method comprising:
obtaining a sample comprising a nucleic acid template (para [0014], "analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample)", wherein "target nucleic acid" is synonymous with "nucleic acid template");
tiling a plurality of molecular inversion probes across a portion of the template (para [0123], "FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout showing ten captured sub-targets tiled across a single target. Each position in the target is covered by three sub-targets such that MIP [molecular inversion probe] performance per base pair is averaged across three probes"),
wherein immediately adjacent probes hybridize to the same strand of the nucleic acid template and probes on the same strand hybridize to the template in an overlapping manner (claim 1, "each probe set comprises a plurality of different probes, each probe having a central region flanked by a 5' region and a 3' region that are complementary to nucleic acids flanking the same strand of one of a plurality of subregions of the target nucleic acid, wherein the subregions of the target nucleic acid are different, and wherein each subregion overlaps with at least one other subregion"; para [0123], "FIG. 1 illustrates a non-limiting embodiment of a tiled probe layout showing ten captured sub-targets tiled across a single target. Each position in the target is covered by three sub-targets such that MIP performance per base pair is averaged across three probes");
filling-in a region between targeting arms of a plurality of the molecular inversion probes with nucleotides (para [0339]-[0354], Example 6, "[0347] Convert Hybridized Probes into Covalently-Closed Circular Products Containing Target Nucleic Acids [0348] Prepare fill-in/ligation reaction mixture: ... [0350] 2.5 ul 10.times. Ampligase buffer (Epicentre) [0351] 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems) [0352] 12.5 ul 5 U/ul Ampligase (Epicentre) [0353] 4.75 ul dH2O [0354] Add 1.0 ul of this mix to the hybridized probe reaction, and incubate at 60 C for 10 hours"); and
analyzing the filled-in region of a plurality of the probes to obtain sequence information about the nucleic acid template (para [0376]-[0381], "[0376] Prepare a Shotgun Next-Generation Sequencing Library for Analysis... [0379] Mechanically shear, using either a nebulizer, BioRuptor, Hydroshear, Covaris, or similar instrument. DNA should be sheared into fragments several hundred basepairs in length. [0380] Ligate adapters required for amplification by the sequencing platform used... [0381] Perform Sequencing of Library According to Manufacturer's Directions (e.g. Illumina, ABI, etc), Reading the Target Sequence to Determine Abundance of the Target Nucleic Acid").
Porreca does not specifically disclose that immediately adjacent probes hybridize to opposite strands of the nucleic acid template. However, Porreca does disclose selecting molecular inversion probes targeting opposing strands of the DNA target (para [0171], "In some embodiments, targeting arms that can be used for capturing the repeat region of the Fragile X locus can have the following sequences or complementary to these sequences depending on the strand that is captured"). Thus, while Porreca does not specifically disclose the avoidance of probe interference by selecting alternate inversion probes so as to target opposing strands of the DNA target in alternation, it would have been obvious to one of ordinary skill in the art that genetic information is present in two equivalent complementary copies, which can be separately detected by MIP probes. It would have been further obvious to one of ordinary skill in the art that by utilizing MIPs hybridizing to both, sense and anti-sense, strands of the target nucleic acid the density of accessible for hybridization DNA target sequences would increase by up to a 100%.

As to claim 2, Porreca discloses that analyzing is by sequencing (para [0295], "Perform Sequencing of Library According to Manufacturer's Directions (e.g. Illumina, ABI, etc), Reading Both the Target Sequence and the Differentiator Tag Sequence").

As to claim 3, Porreca discloses amplifying the filled-in region of the plurality of the probes prior to sequencing (para [0276], "Amplify Circular Material by PCR Using Primers Specific to the 'Constant Region' of the Probes").

********** See Supplemental Sheet to continue **********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.: PCT/US 14/27324

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V(2). Citations and Explanations:

As to claims 6 and 7, Porreca discloses sequencing information comprises a mutation in the nucleic acid sequence, wherein the mutation is selected from the group consisting of: a single nucleotide polymorphism (SNP), and insertion, a deletion, an inversion, a translocation, a substitution, and a combination thereof (para [0133], "Multiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs, or preparatively, often for next-generation sequencing or other sequencing techniques" para [0155], "The mutations listed in Table 2 are documented polymorphisms in several disease-associated genes... The polymorphisms are of several types: insertion/deletion polymorphisms which will cause frameshifts (and thus generally interrupt protein function) unless the insertion/deletion length is a multiple of 3 bp, and substitutions which can alter the amino acid sequence of the protein and in some cases cause complete inactivation by introduction of a stop codon").

As to claim 8, Porreca discloses that the filling-in step uses a ligase (para [0220], "Prepare fill-in/ligation reaction mixture:... [0222] 2.5 ul 10.times. Ampligase buffer (Epicentre) [0223] 5.0 ul 5 U/ul Taq Stoffel fragment (Applied Biosystems) [0224] 12.5 ul 5 U/ul Ampligase (Epicentre)").

As to claim 9, Porreca discloses that the filling-in step uses a polymerase (para [0235] "Prepare PCR mixture: ... [0240] 0.4 ul 2.5 U/ul Accuprime Pfx polymerase (Invitrogen)").

As to claim 10, Porreca discloses that a ligase is used to covalently link the filled-in region to one of the targeting arms (para [0219], "Convert Hybridized Probes into Covalently-Closed Circular Products Containing Subtargets [0220] Prepare fill-in/ligation reaction mixture:... [0222] 2.5 ul 10.times. Ampligase buffer (Epicentre)... [0224] 12.5 ul 5 U/ul Ampligase (Epicentre)").

As to claim 11, Porreca discloses that prior to sequencing, a barcode is attached to the molecular inversion probe (para [0149], "barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together"; para [0198], "In some embodiments, a sequencing technique (e.g., a next-generation sequencing technique) is used to sequence part of one or more captured targets (e.g., or amplicons thereof) and the sequences are used to count the number of different barcodes that are present. Accordingly, in some embodiments, aspects of the invention relate to a highly-multiplexed qPCR reaction", para [0175] "When a precise measurement of the length of both alleles from a diploid sample is desired, further manipulations may be required. This is because the capture efficiency measured will actually be the average efficiency of the two alleles. To effectively achieve separate measurements for each allele, barcodes (e.g., sequence tags) can be used that allow the efficiency of individual capture events (from individual genomic loci) to be followed. FIG. 11A-C shows the approach... The allele lengths can therefore be read directly off this graph, after absolute length calibration using known standards. In some embodiments, a sequencing technique (e.g., a next-generation sequencing technique) is used to sequence part of one or more captured targets (e.g., or amplicons thereof) and the sequences are used to count the number of different barcodes that are present. Accordingly, in some embodiments, aspects of the invention relate to a highly-multiplexed qPCR reaction").

As to claim 12, Porreca discloses that prior to sequencing, a sequencing adaptor is attached to the molecular inversion probe (para [0150], "The sequencing primers must be incorporated subsequently such that they sequence both the differentiator tag sequence and the target sequence. Alternatively, the sequencing adaptors can be synthesized with the random differentiator tag sequences at their 3' end (as degenerate bases), so that only one ligation must be performed. Another method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags").

As to claim 13, Porreca discloses the method according to claim 2, wherein prior to sequencing, a barcode and a sequencing adaptor are attached to the molecular inversion probe (para [0150], "The actual incorporation of the random differentiator tag sequences can be performed through various methods known in the art. For example, nucleic acids comprising differentiator tag sequences may be incorporated by ligation. This is a flexible method, because molecules having differentiator tag sequence can be ligated to any blunt-ended nucleic acids. The sequencing primers must be incorporated subsequently such that they sequence both the differentiator tag sequence and the target sequence. Alternatively, the sequencing adaptors can be synthesized with the random differentiator tag sequences at their 3' end (as degenerate bases), so that only one ligation must be performed. Another method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags").

Claims 4 and 5 lack an inventive step under PCT Article 33(3) as being obvious over Porreca, as above, in view of an article titled "The properties and applications of single-molecule DNA sequencing" by Thompson, et al. (hereinafter "Thompson").

As to claims 4 and 5, Porreca discloses analyzing of the nucleic acid template by sequencing (para [0295], "Perform Sequencing of Library According to Manufacturer's Directions (e.g. Illumina, ABI, etc), Reading Both the Target Sequence and the Differentiator Tag Sequence"), but does not specifically disclose that sequencing is done by sequencing-by-synthesis. Thompson discloses using single molecule sequencing-by-synthesis for high throughput analysis of nucleic acids (pg 5, Fig 2 and its legend, "Overview of single-molecule sequencers. The three most advanced single-molecule sequencing systems all carry out sequencing-by-synthesis using laser excitation to generate a fluorescent signal from labeled nucleotides, which is then detected using a camera") and further discloses that sequencing-by-synthesis technique is advantageous because it avoids "the many artifacts associated with reverse transcriptase and providing unparalleled quantitative accuracy" (pg 3, col 2). It would have been obvious to one of ordinary skill in the art at the time of the invention to combine Porreca and Thompson by incorporating, in the course of routine experimentation and with a reasonable expectation of success, single molecule sequencing-by-synthesis disclosed by Thompson into the method of Porreca, to improve sensitivity and accuracy of the method of analyzing of the nucleic acid template disclosed by Porreca.

********** See the Following Supplemental Sheet to continue **********

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.: PCT/US 14/27324

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of:
Box V(2). Citations and Explanations and the Preceding Supplemental Sheet:

Claims 14 and 15 lack an inventive step under PCT Article 33(3) as being obvious over Porreca, as above, in view of US 2008/0269068 A1 to Church, et al. (hereinafter "Church").

As to claim 14, Porreca does not specifically disclose that prior to sequencing, the molecular inversion probe is attached to a solid surface. Church, in the context of a "method of analyzing an array of nucleic acid sequences" (Church, claim 1), discloses that prior to sequencing, the molecular inversion probe is attached to a solid surface (Church, claim 1, "b) providing a plurality of molecular inversion probes, each molecular inversion probe having a tag sequence, a barcode sequence, and two guide sequences; c) hybridizing the molecular inversion probes to the immobilized query oligonucleotide sequences; d) performing rolling circle amplification such that the barcode sequence of one molecular inversion probe is transferred to one immobilized query oligonucleotide sequence; e) arraying the immobilized query oligonucleotide sequences; and f) identifying barcodes present on an immobilized query oligonucleotide sequence"; claims 6, 7, "the plurality of immobilized query oligonucleotide sequences are immobilized on beads...the beads are arranged on a solid support"). It would have been obvious to one of ordinary skill in the art at the time of the invention to combine Porreca and Church by incorporating, in the course of routine experimentation and with a reasonable expectation of success, immobilization of the molecular inversion probe on a solid surface prior to sequencing, to facilitate analysis of the resulting sequencing reads.

As to claim 15, Porreca in view of Church discloses that the surface is a surface of a flow cell (Church, para [0081]-[0083], Example III, "[0083] 16 query adaptors will be ligated with all possible two nucleotide 3' overhangs, with 4 different 16-mer sequences associable with the identity of the 5'-most query base (magenta) (FIG. 7, Step 1). 160 different possible pairings of 40 EcoP15I and 4 query 16-mers, separated by 17-26 base pairs, should be present on every bead. Non-biotinylated strands will be denatured and washed away. The remaining strands will be immobilized and loaded into a flow cell (FIG. 7, Step 2). No further enzymology is necessary after this point. 40 four-color hybridization-imaging cycles will be performed to read four contiguous 10-mer tag sequences").

Claims 1-15 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Porreca et al.	ART UNIT:	1637
SERIAL NUMBER:	13/081,660	CONFIRMATION NO.:	4441
FILING DATE:	April 7, 2011	EXAMINER:	Strzelecka, Teresa E.
TITLE:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION		

FILED ELECTRONICALLY

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

RESPONSE

This paper is in response to the Office Action mailed September 11, 2014 from the United States Patent and Trademark Office. Applicants believe no fee is due with this response, but nevertheless applicants authorize the Commissioner to charge any required fees due to Deposit Account No. 50-0369.

Amendments to the claims begin on page 2.

Remarks begin on page 9.

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

1. (Previously Presented) A method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction, the method comprising:

obtaining a template nucleic acid;

incorporating at least two of the same sequence identifiers into the template;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the two incorporated sequence identifiers remain associated with the template, thereby validating the integrity of the nucleic acid template in a multiplex sequencing reaction.

2. (Original) The method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

3. (Original) The method according to claim 2, wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

4. (Withdrawn) The method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

5-6. (Canceled)

7. (Original) The method according to claim 1, wherein prior to sequencing, the template is attached to a substrate.

8. (Original) The method according to claim 2, wherein the template is directly attached to the substrate.

9. (Withdrawn) The method according to claim 2, wherein the template is indirectly attached to the substrate.

10. (Original) The method according to claim 7, further comprising, amplifying the template.

11. (Original) The method according to claim 1, wherein sequencing is sequencing by synthesis.

12. (Original) The method according to claim 11, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

13. (Original) The method according to claim 1, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

14. (Original) The method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

15. (Original) The method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

16. (Original) The method according to claim 1, wherein the pair of identifiers are first and second barcode sequences that are attached to the template.

17. (Withdrawn) The method according to claim 16, wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template.

18. (Original) The method according to claim 17, wherein the first and second barcode sequences are the same.

19. (Withdrawn) The method according to claim 17, wherein the first and second barcode sequences are different.

20. (Previously presented) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a pair of the same barcode sequences to the template;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

21. (Previously presented) The method according to claim 20, wherein a first barcode sequence is incorporated into a 5' portion of the template and a second barcode sequence is incorporated into a 3' portion of the template.

22. (Original) The method according to claim 21, wherein after attaching of the pair of barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

23. (Withdrawn) The method according to claim 20, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

24. (Original) The method according to claim 20, wherein prior to sequencing, the template is attached to a substrate.

25. (Original) The method according to claim 24, wherein the template is directly attached to the substrate.

26. (Withdrawn) The method according to claim 24, wherein the template is indirectly attached to the substrate.

27. (Original) The method according to claim 24, further comprising, amplifying the template.

28. (Original) The method according to claim 20, wherein sequencing is sequencing by synthesis.

29. (Original) The method according to claim 28, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

30. (Original) The method according to claim 20, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

31. (Previously presented) The method according to claim 30, wherein the labeled nucleotide is an optically labeled nucleotide.

32. (Original) The method according to claim 31, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

33-34. (Canceled)

35. (Previously Presented) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template, wherein the first and second barcode sequences are the same;

amplifying the template;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

36. (Original) The method according to claim 35, wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

37. (Withdrawn) The method according to claim 35, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

38. (Original) The method according to claim 35, wherein prior to amplifying, the template is immobilized to a solid support.

39-40. (Canceled)

41. (Original) The method according to claim 35, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

42. (Withdrawn) A method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte.

43. (Withdrawn) The method of claim 42, wherein said identifiers are nucleic acid sequence tags.

44. (Withdrawn) The method of claim 43, wherein said sequence tags are associated with a moiety that binds said analyte.

45. (Withdrawn) The method of claim 42, wherein said analyte is a protein.

46. (Withdrawn) The method of claim 45, wherein said identifiers are peptides.

Remarks

Claims 1-4, 7-32, 35-38, and 41-46 remain pending in the application. No claims are amended and no new matter is added.

Rejection under 35 U.S.C. §102(b)

The Office Action maintains the rejection of claims 1-3, 7-8, 10-12, 16, 20-22, 24-25, 27-29, 35-36, and 38 under 35 U.S.C. §102(b) in view of Parameswaran (Nucl. Acids. Res., 35:e130, pp. 1-9, 2007). The Examiner appears to maintain this rejection based on the allegation that, because the term "barcode" is not defined in the application, it can be interpreted to mean any sequence of two or more nucleotides. Applicants respectfully disagree.

The legal standard for a novelty rejection is that the Patent Office must provide a single prior art reference that discloses each and every element of a claim. M.P.E.P. §2131. The claims recite incorporating at least two of the same sequence identifiers into the template. A claim requiring the incorporation of two of the same sequence identifiers is novel over Parameswaran, because that reference specifically requires attaching barcodes that are not the same. Parameswaran describes its barcode design as follows:

> Sets of barcodes have been independently designed for the forward PCR primers (FORWARD barcodes) and the reverse PCR primers (REVERSE barcodes) used in library construction (Figure 1). Length of the barcode was chosen to allow for definitive assignment of barcodes, while minimizing the number of base reads used per barcode. The barcode design (Table 1) facilitated unambiguous assignment, with care taken to avoid homopolymeric runs that are particularly problematic in pyrosequencing technologies. All forward barcodes were of the type HIJJK LLMNO, while reverse barcodes were of the type HIJKK LMMNO. H, I, J, K and L, M, N, O are uniquely partnered with one of four nucleotides: A, C, G and T. The barcodes thus contained two instances of a 1–2 base run of each nucleotide (one from the H, I, J, K set, and the other from the L, M, N, O set), with no more than two successive occurrences of the same nucleotide (Table 1). This design avoids significant problems due to error rate at homopolymeric

> runs of nucleotides. The forward and reverse barcodes varied in dinucleotide positioning along the length of the barcode. Positions 3, 4 (nucleotide J) and 6, 7 (nucleotide L) were homopolymeric dinucleotides in the forward barcode, while positions 4, 5 (nucleotide K) and 7, 8 (nucleotide M) were homopolymeric dinucleotides in the reverse barcode. Parameswaran at 4.

The barcodes follow a particular formula that guarantees the forward and reverse sequences will be different from each other. Parameswaran specifies that "any two forward or reverse barcodes [differ] in at least 4nt . . . . These serve as strong unique identifiers for each of the forward and reverse barcode sets" *Id*. Regardless of the specific composition, Parameswaran clearly requires attaching different barcodes. In contrast, the present claims expressly require incorporation of two of the same sequence identifiers, regardless of the composition of those two sequence identifiers. For example, even if the sequence identifier was only two nucleotides, those two nucleotides would need to be the same for both sequence identifiers. That is not possible according to Parameswaran, which requires barcodes with at least four nucleotides that are different. Therefore, regardless of the definition of the term "barcode," the claims are novel in view of Parameswaran, because that reference requires different barcodes and the pending claims require incorporation of two of the same sequence identifiers.

Accordingly, it is not possible for Parameswaran to be an anticipatory reference to the pending claims and Applicants respectfully request withdrawal of the 35 U.S.C. §102(b) rejection.

Rejection under 35 U.S.C. §103(a)

The Office Action rejects claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, and 41 under 35 U.S.C. §103(a) in view of the combination of Kawashima (U.S. patent application publication number 2005/0100900), Parameswaran, and Kircher et al. (Bioessays, 32:524-536, 2010). This rejection appears to be based on the disclosure in paragraph [00183] and FIGS. 13 and 15 of Kawashima. Applicants respectfully disagree.

In order to establish a prima facie case of obviousness, three basic criteria must be established by the Patent Office. First, there must be a suggestion in the cited art to modify a reference or to combine multiple references in a manner that would lead one of ordinary skill in the art to an applicant's claimed invention. Second, the cited references themselves must convey a reasonable expectation of success in arriving at the claimed invention based upon the proposed modification or combination. Finally, the teaching or suggestion to make the claimed invention must be found in the prior art and not in the applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). In addition to the requirements for making a combination, an obviousness rejection cannot be established in the first place unless each claim element is disclosed in the cited art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

While Kawashima reports that different tags can be used for different samples (Kawashima at paragraph [0183]), Kawashima does not disclose or suggest that templates from the same strand would have the same tags, i.e., incorporation of two of the same sequence identifiers into a template nucleic acid. In fact, Kawashima is silent regarding attaching more than one of the same tags to a nucleic acid template. The only mention of tags in Kawashima refer to tags being different for each sample.

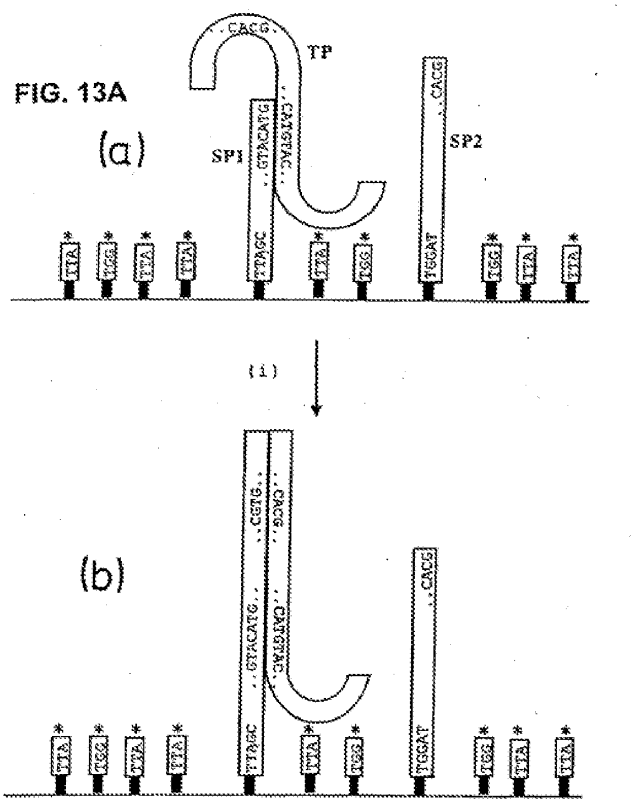
The Examiner points to FIG. 15b, which indicates the presence of "tags." But that figure merely shows more than one tag sequence being used per nucleic acid template. The figure is silent as to whether the tags are the same. Furthermore, FIG. 15 is not described anywhere in the specification, and so no clarification of the "tags" is provided.

When tags are described in the specification, they are described as different tags being used for different samples. Paragraphs [0181]-[0189] are the only place in Kawashima that describe the tags with any precision. For example, Kawashima states:

> the oligonucleotide linkers used to tail the randomly cut genomic DNA fragments are . . . made of two parts: the sequence of the primers grafted onto the surface (P1 and P2, FIG. 13b) and a "tag" sequence which is different for

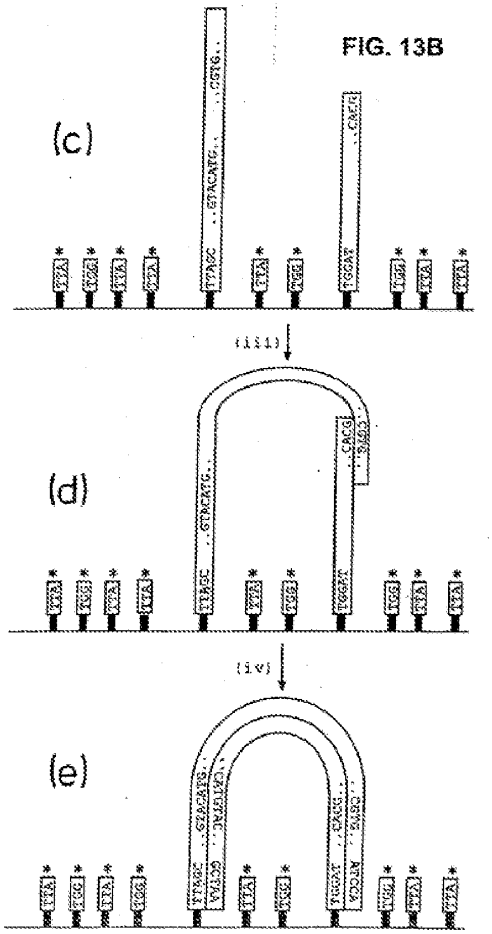
each sample and which will be used for identifying the origin of the DNA colony. Note that for each sample, the tag may not be unique, but a plurality of tags could be used. Kawashima, para. [0183].

FIGs. 13a-b, which paragraph [00183] references, show a template including two different tag sequences. That figure is reproduced below:



Figs. 13a-b show one template sequence that has two tags. The first tag has a sequence of CATGTAC, while a second tag has a sequence of CACG. Clearly, those two tags are different, and therefore paragraph [00183] describes an approach in which two different tags are incorporated into a template sequence.

FIGs. 13c-e show a nucleotide sequence again having two distinct tags: GTACATG and CGTG. Figs. 13c-e are reproduced below:



Again, the first tag has a sequence of CATGTAC, while a second tag has a sequence of CACG. Clearly, those two tags are different, and therefore Figs. 13c-e also describe an approach in which two different tags are incorporated into a template sequence.

The description in Figs. 13a-e is the only place in the specification where the composition of the tags are described.

In short, although Kawashima reports a plurality of tags, nowhere does Kawashima disclose or suggest attaching two or more of the same tag to a sample, and every time Kawashima shows the actual sequence of the tags, they are different from each other.

Accordingly, Kawashima fails to disclose or suggest incorporating two of the same sequence identifiers into a template nucleic acid. None of the other cited references cure that deficiency of Kawashima.

As discussed above, Parameswaran is limited to reporting incorporation of different barcode sequences into a single template. Kircher is a review article limited to reporting different types of sequencing platforms. Kircher fails to disclose or suggest any type of identifier, let alone a method that involves incorporating at least two of the same sequence identifiers into a nucleic acid template.

Accordingly, the amended claims are not obvious in view of any combination of Kawashima, Parameswaran, and Kircher, because those references fail to disclose or suggest the element of incorporating at least two of the same sequence identifiers into a nucleic acid template.

Applicants respectfully request withdrawal of the 35 U.S.C. §103(a) rejection.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: December 11, 2014

Respectfully submitted,
BROWN RUDNICK LLP

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/
Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

61810308

Electronic Acknowledgement Receipt	
EFS ID:	20929508
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Catherine Heath
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	11-DEC-2014
Filing Date:	07-APR-2011
Time Stamp:	14:11:46
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment/Req. Reconsideration-After Non-Final Reject	GSGE00101US2925814Resonse.pdf	163579 12e3137281899dc6055ecb043540b66f38d414ba	no	15
Warnings:					
Information:					

Total Files Size (in bytes):	163579

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National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/081,660	Filing Date 04/07/2011	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	12/11/2014	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 40	Minus	** 46	= 0	X $40 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/DIANE FLOYD/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6462254		2002-10-08	Vernachio et al.	
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	1	2006/084132	WO	A2	2006-08-10	Agencourt Bioscience Corp		☐
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NON-PATENT LITERATURE DOCUMENTS			Remove
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

	1	English translation of Notice of Reasons for Rejection mailed on November 13, 2014, for Japanese Patent Application No. 2013-546224, corresponding to International Patent Application Serial No. PCT/US2011/065098, with International Filing Date December 15, 2011 (8 pages)	☐
	2	Meyer et al., 2007, Targeted high-throughput sequencing of tagged nucleic acid samples, Nucleic Acids Research 35 (15):e97 (5 pages).	☐
	3	Smith et al., 2010, Highly-multiplexed barcode sequencing: an efficient method for parallel analysis of pooled samples, Nucleic Acids Research 38(13):e142 (8 pages).	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2014-12-22
Name/Print	Thomas C. Meyers	Registration Number	36989

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal	
Application Number:	13081660
Filing Date:	07-Apr-2011
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Filer:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Filed as Small Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	2806	1	90	90
		Total in USD ($)		**90**

Electronic Acknowledgement Receipt	
EFS ID:	21022613
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers
Filer Authorized By:	
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	22-DEC-2014
Filing Date:	07-APR-2011
Time Stamp:	13:12:36
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$90
RAM confirmation Number	13366
Deposit Account	500369
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

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1	Information Disclosure Statement (IDS) Form (SB08)	Information_Disclosure_Statement_Fillable_PDF.PDF	612642 7b0e1b5c49afb9f32a758baf0963c3ecc9f5c8a5	no	4
Warnings:					
Information:					
2	Non Patent Literature	JP2013-546224-translation-Notice-Reasons-Rejection.PDF	351126 8649f8f537803632586e1a1e65b03114a4efaa06	no	8
Warnings:					
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3	Non Patent Literature	Meyer-2007-NAR.PDF	1154866 36c47fdd6ea4a03ee2bba690733b57ba3b56d0b8	no	5
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Warnings:					
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Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	30887 40ed0469517b324d9b59d4cf8268ab14849be069	no	2
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

21710 7590 01/05/2015

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

NOTIFICATION DATE	DELIVERY MODE
01/05/2015	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ip@brownrudnick.com
usactions@brownrudnick.com

Office Action Summary	Application No. 13/081,660	Applicant(s) PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12/11/14*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-4,7-32,35-38 and 41-46* is/are pending in the application.

5a) Of the above claim(s) *4,9,17-19,23,26,37 and 42-46* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-3,7,8,10-16,20-22,24,25,27-32,35,36,38 and 41* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *10/15/14;12/22/14*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

1. The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

2. This office action is in response to an amendment filed December 11, 2014. Claims 1-4, 7-32, 35-38 and 41-46 were previously pending, with claims 4, 9, 17-19, 23, 26, 37 and 42-46 withdrawn from consideration. Applicant did not amend any claims. Claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38 and 41 are under examination.

3. Applicant's arguments did not overcome any of the previously presented rejections for reasons given in the "Response to Arguments" below.

Information Disclosure Statement

4. The information disclosure statements (IDSs) submitted on October 15, 2014 and December 22, 2014 were filed after the mailing date of the non-final office action on September 11, 2014. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

Response to Arguments

5. Applicant's arguments filed December 11, 2014 have been fully considered but they are not persuasive.

A) Regarding the rejection of claims 1-3, 7, 8, 10-12, 16, 20-22, 24, 25, 27-29, 35, 36 and 38 under 35 U.S.C. 102(b) over Parameswaran et al. Applicant argues the following:

"The legal standard for a novelty rejection is that the Patent Office must provide a single prior art reference that discloses each and every element of a claim. M.P.E.P. §2131. The claims recite incorporating at least two of the same sequence identifiers into the template. A claim requiring the incorporation of two of the same sequence identifiers is novel over Parameswaran,

because that reference specifically requires attaching barcodes that are not the same.

Parameswaran describes its barcode design as follows:

> Sets of barcodes have been independently designed for the forward PCR primers (FORWARD barcodes) and the reverse PCR primers (REVERSE barcodes) used in library construction (Figure 1). Length of the barcode was chosen to allow for definitive assignment of barcodes, while minimizing the number of base reads used per barcode. The barcode design (Table 1) facilitated unambiguous assignment, with care taken to avoid homopolymeric runs that are particularly problematic in pyrosequencing technologies. All forward barcodes were of the type HIJJK LLMNO, while reverse barcodes were of the type HIJKK LMMNO. H, I, J, K and L, M, N, O are uniquely partnered with one of four nucleotides: A, C, G and T. The barcodes thus contained two instances of a 1-2 base run of each nucleotide (one from the H, I, J, K set, and the other from the L, M, N, O set), with no more than two successive occurrences of the same nucleotide (Table 1). This design avoids significant problems due to error rate at homopolymeric runs of nucleotides. The forward and reverse barcodes varied in dinucleotide positioning along the length of the barcode. Positions 3, 4 (nucleotide J) and 6, 7 (nucleotide L) were homopolymeric dinucleotides in the forward barcode, while positions 4, 5 (nucleotide K) and 7, 8 (nucleotide M) were homopolymeric dinucleotides in the reverse barcode. Parameswaran at 4.

The barcodes follow a particular formula that guarantees the forward and reverse sequences will be <u>different</u> from each other. Parameswaran specifies that "any two forward or reverse barcodes [differ] in at least 4nt ....These serve as strong unique identifiers for each of the forward and reverse barcode sets" *Id.* Regardless of the specific composition, Parameswaran clearly requires attaching <u>different</u> barcodes. In contrast, the present claims expressly require incorporation of two of the same sequence identifiers, regardless of the composition of those two sequence identifiers. For example, even if the sequence identifier was only two nucleotides, those two nucleotides would need to be the same for both sequence identifiers. That is not possible according to Parameswaran, which requires barcodes with at least four nucleotides that are different. Therefore, regardless of the definition of the term "barcode," the claims are novel in

view of Parameswaran, because that reference requires different barcodes and the pending claims require incorporation of two of the same sequence identifiers.

Accordingly, it is not possible for Parameswaran to be an anticipatory reference to the pending claims and Applicants respectfully request withdrawal of the 35 U.S.C. § 102(b) rejection."

However, Applicant did not define the term "sequence identifier" or "barcode", therefore, these terms are interpreted as a sequence of two or more nucleotides. The claims do not require barcodes of any specific length or sequence, either. Therefore, as stated in the rejection, when two-nucleotide sequence is considered as a sequence identifier, Parameswaran et al. anticipate the claims.

The rejection is maintained.

B) Regarding the rejection of claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, 39 and 41 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kawashima et al., Parameswaran et al. and Kircher et al., Applicant argues the following:

"While Kawashima reports that different tags can be used for different samples (Kawashima at paragraph [0183]), Kawashima does not disclose or suggest that templates from the same strand would have the same tags, i.e., incorporation of two of the same sequence identifiers into a template nucleic acid. In fact, Kawashima is silent regarding attaching more than one of the same tags to a nucleic acid template. The only mention of tags in Kawashima refer to tags being different for each sample.

The Examiner points to FIG. 15b, which indicates the presence of "tags." But that figure merely shows more than one tag sequence being used per nucleic acid template. The figure is silent as to whether the tags are the same. Furthermore, FIG. 15 is not described anywhere in the specification, and so no clarification of the "tags" is provided.

When tags are described in the specification, they are described as different tags being used for different samples. Paragraphs [0181]- [0189] are the only place in Kawashima that describe the tags with any precision. For example, Kawashima states:

> the oligonucleotide linkers used to tail the randomly cut genomic DNA fragments are.., made of two parts: the sequence of the primers grafted onto the surface (P1 and P2, FIG. 13b) and a "tag" sequence which is different for each sample and which will be used for identifying the origin of the DNA colony. Note that for each sample, the tag may not be unique, but a plurality of tags could be used. Kawashima, para. [0183].

FIGs. 13a-b, which paragraph [00183] references, show a template including two different tag sequences. That figure is reproduced below:

(Figure omitted)

Figs. 13a-b show one template sequence that has two tags. The first tag has a sequence of CATGTAC, while a second tag has a sequence of CACG. Clearly, those two tags are different, and therefore paragraph [00183] describes an approach in which two different tags are incorporated into a template sequence.

FIGs. 13c-e show a nucleotide sequence again having two distinct tags: GTACATG and CGTG. Figs. 13c-e are reproduced below:

(Figures omitted)

Again, the first tag has a sequence of CATGTAC, while a second tag has a sequence of CACG. Clearly, those two tags are different, and therefore Figs. 13c-e also describe an approach in which two different tags are incorporated into a template sequence.

The description in Figs. 13a-e is the only place in the specification where the composition of the tags are described.

In short, although Kawashima reports a plurality of tags, nowhere does Kawashima disclose or suggest attaching two or more of the same tag to a sample, and every time Kawashima shows the actual sequence of the tags, they are different from each other.

Accordingly, Kawashima fails to disclose or suggest incorporating two of the same sequence identifiers into a template nucleic acid. None of the other cited references cure that deficiency of Kawashima.

As discussed above, Parameswaran is limited to reporting incorporation of different barcode sequences into a single template. Kircher is a review article limited to reporting different types of sequencing platforms. Kircher fails to disclose or suggest any type of identifier, let alone a method that involves incorporating at least two of the same sequence identifiers into a nucleic acid template.

Accordingly, the amended claims are not obvious in view of any combination of Kawashima, Parameswaran, and Kircher, because those references fail to disclose or suggest the

element of incorporating at least two of the same sequence identifiers into a nucleic acid template.

Applicants respectfully request withdrawal of the 35 U.S.C. § 103(a) rejection."

However, as explained above, the claims do not require any specific barcode sequences or lengths, and Applicant did not define the term "sequence identifier" or "barcode", therefore, these terms are interpreted as a sequence of two or more nucleotides. Therefore Fig. 15 of Kawashima et al. clearly shows the same tag on both 5' and 3' ends of a target. Further, regarding the tags in Fig. 13a-b, the first sequence "CATGTAC" has two-nucleotide sequences of "CA" and "AC", as does the second sequence "CACG", therefore both of these sequences comprise identical two-nucleotide tags. Similarly, the sequence "GTACATG" in Fig. 13 c-e contains two-nucleotide sequences of "GT" and "TG", as does the second sequence "CGTG", therefore both of these sequences comprise identical two-nucleotide tags.

The rejection is maintained.

Claim Interpretation

6. Applicant did not define the term "sequence identifier" or "barcode", therefore any sequence of two or more nucleotides is interpreted as a "sequence identifier" or a "barcode".

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 1-3, 7, 8, 10-12, 16, 20-22, 24, 25, 27-29, 35, 36 and 38 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Parameswaran et al. (Nucl. Acids Res., vol. 35, e130, pp. 1-9, 2007; previously cited).

Claims 1, 2, 10, 16, 20, 21, 27 and 35 are considered together in claim 35, since it contains limitations of all of these claims.

Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Parameswaran et al. teach a method comprising:

obtaining a nucleic acid template (page 2, third paragraph);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template, wherein the first and second barcode sequences are the same (page 2, paragraphs 4-6; page 3; page 4, first and second paragraphs; Fig. 1. As can be seen from the supplementary Table 1A, for sample 41, for example, the sequences AGC and GCT in the barcode part of the primer are the same in the forward and reverse primers. Therefore Parameswaran et al. teach barcodes with the same sequences.);

amplifying the template (page 4, third paragraph);

sequencing the template to generate sequence reads (page 4, third paragraph), and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with a template (page 7, paragraphs 2-4; page 8, first and second paragraph; Table 3).

Regarding claims 3, 22 and 36, Parameswaran et al. teach the template having the following configuration: 5'-F-adapter (=first known sequence)- F-barcode (=a first barcode sequence)-F-cloning linker (=a second known sequence)-target sequence-RC (R-cloning linker) (=a third known sequence)-RC (R-barcode) (= a second barcode sequence)-RC(R-adapter) (= a fourth known sequence) (Fig. 1).

Regarding claims 7, 8, 24, 25 and 38, Parameswaran et al. teach the template undergoing emulsion PCR, therefore they inherently teach template directly immobilized on a solid support (page 4, third paragraph).

Regarding claims 11, 12, 28 and 29, Parameswaran et al. teach pyrosequencing (page 4, third paragraph), therefore they inherently teach single molecule sequencing by synthesis, since each DNA molecule is sequenced individually.

Claim Rejections - 35 USC § 103

9. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38 and 41 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Kawashima et al. (US 2005/0100900 A1; published May 2005; previously cited), Parameswaran et al. (Nucl. Acids Res., vol. 35, e130, pp. 1-9, 2007; previously cited) and Kircher et al. (Bioessays, vol. 32, pp. 524-536, June 2010; previously cited).

A) Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Kawashima et al. teach a method comprising:

obtaining a nucleic acid template (page 4, [0049]-[0051]; [0056]);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template, wherein the first and second barcode sequences are the same (page 2, [0026]-[0029]; page 4, [0052]-[0053]; Fig. 15, page 13, [0182]-[0189], where the tag sequences are the barcodes, and the tags are the same as indicated in Fig. 15d, Fig. 16b and where the tag identifies a sample, but not the nucleic acid strand);

amplifying the template (Fig. 1; page 6, [0109]; page 7, [0110]-[0124]; page 10, [0149]-[0157]); and

sequencing the template (Fig. 17; page 15-18).

Regarding claims 3, 22 and 36, Kawashima et al. teach the template having the following configuration: 5'-P1 primer (=first known sequence)- tag (=a first barcode sequence)-S1 (=a second known sequence)-target sequence-S2 (=a third known sequence)-tag (= a second barcode sequence)-P2 primer (= a fourth known sequence) (Fig. 15d, Fig. 16b).

Regarding claims 7, 8, 24, 25 and 38, Kawashima et al. teach the template directly immobilized on a solid support (Fig. 1).

Regarding claims 11, 12, 28 and 29, Kawashima et al. teach single molecule sequencing by synthesis (Fig. 17, page 5, [0067]-[0075]; page 15, [0233]-[0238]).

Regarding claims 13, 30 and 41, Kawashima et al. teach hybridizing a primer to the template, contacting the duplex with a polymerase in the presence of detectably labeled

nucleotide, detecting the signal from incorporated nucleotide and repeating the process to determine the sequence of nucleic acid (Fig. 17; page 15, [0238]-[0245]).

Regarding claims 14, 15, 31 and 32, Kawashima et al. teach fluorescently labeled nucleotides (page 15, [0239]-[0241]).

B) Kawashima et al. do not teach that the barcodes can be used to validate the integrity of nucleic acid templates.

C) Regarding claims 1, 2, 10, 16, 20, 21, 27 and 35, Parameswaran et al. teach a method comprising:

obtaining a nucleic acid template (page 2, third paragraph);

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template (page 2, paragraphs 4-6; page 3; page 4, first and second paragraphs; Fig. 1);

amplifying the template (page 4, third paragraph);

sequencing the template to generate sequence reads (page 4, third paragraph), and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with a template (page 7, paragraphs 2-4; page 8, first and second paragraph; Table 3).

Regarding claims 3, 22 and 36, Parameswaran et al. teach the template having the following configuration: 5'-F-adapter (=first known sequence)- F-barcode (=a first barcode sequence)-F-cloning linker (=a second known sequence)-target sequence-RC (R-cloning linker) (=a third known sequence)-RC (R-barcode) (= a second barcode sequence)-RC(R-adapter) (= a fourth known sequence) (Fig. 1).

Regarding claims 7, 8, 24, 25 and 38, Parameswaran et al. teach the template undergoing emulsion PCR, therefore they inherently teach template directly immobilized on a solid support (page 4, third paragraph).

Regarding claims 11, 12, 28 and 29, Parameswaran et al. teach pyrosequencing (page 4, third paragraph), therefore they inherently teach single molecule sequencing by synthesis, since each DNA molecule is sequenced individually.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the barcodes of Kawashima et al. to validate the integrity of the nucleic acid molecules being sequenced, as suggested by Parameswaran et al. (page 8, last paragraph):

"Our barcode specification has been tailored for use with pyrosequencing technologies, particularly reflecting restrictions imposed on the generation of homopolymeric runs. These barcodes may also be compatible with other sequencing platforms. Optimally, however the precise structure of the barcodes might need to be revised to reflect sequencing error profiles of the various systems. If necessary, multiple sequencing primers may also be used to identify the barcode and the sequence of interest, to overcome limitations imposed by short read lengths. As the availability of high-throughput technology advances, massively multiplexed barcoded sequencing will be extremely useful in performing surveys of diverse systems for nucleic acid signatures."

As stated by Kircher et al. regarding the type of sequencing performed by the method of Kawashima et al. (page 527, last three paragraphs; page 528; Fig. 3):

"The Illumina library and flow cell preparation includes several in vitro amplification steps, which cause a high background error rate and contribute to the average error rate of about 10^{-2}–10^{-3} [41, 42]. Further, the flow cell preparation creates a fraction of ordinary-looking clusters that are initiated from more than one individual sequence. These results in mixed signals and mostly low quality sequences for these clusters. Similar to the 454 ghost wells, the Illumina image analysis may identify chemistry crystals, dust, and lint particles as clusters and call sequences from these. In such cases the resulting sequences typically appear to be of low sequence complexity."

Therefore one of ordinary skill in the art at the time of the invention would have been motivated to use error detection using barcode sequences to verify correctness of sequencing results as suggested by Parameswaran et al. in the method of Kawashima et al. to avoid sequencing errors resulting from sample preparation and processing described by Kircher et al.

11. No claims are allowed.

Conclusion

12. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event,

however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TERESA E STRZELECKA
Primary Examiner
Art Unit 1637

/TERESA E STRZELECKA/
Primary Examiner, Art Unit 1637
December 29, 2014

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99**)		
	Application Number	13081660
	Filing Date	2011-04-07
	First Named Inventor	Gregory Porreca
	Art Unit	1637
	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US 29258/14

U.S.PATENTS

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/TS/	1	1996/019586	WO	A1	1996-06-27	Visible Genetics Inc		☐
/TS/	2	1998/014275	WO	A1	1998-04-09	Intronn Llc		☐
/TS/	3	2004/083819	WO	A2	2004-09-30	Trace Genetics, Inc		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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/TS/	4	2005/003304	WO	A2	2005-01-13	Illumina, Inc		☐

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
/TS/	1	Thompson, et al., 2011, "The properties and applications of single-molecule DNA sequencing," Genome Biology 12(2):217, 10 pages.	☐
/TS/	2	Australian Patent Examination Report No. 1 issued August 12, 2014, for Australian Patent Application No. 2010242073 filed April 30, 2010, 4 pages.	☐
/TS/	3	International Search Report and Written Opinion mailed September 3, 2014, for International Patent Application No. PCT/US2014/027324, filed March 14, 2014, 7 pages.	☐
/TS/	4	Supplementary European Search Report issued August 26, 2014, for European Patent Application No. 12765217.0, filed March 20, 2012, 5 pages.	☐

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Examiner Signature	/Teresa Strzelecka/	Date Considered	12/29/2014

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PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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	Examiner Name	T. E. Strzelecka
	Attorney Docket Number	GSGE-001/01US

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/TS/	1	6462254		2002-10-08	Vernachio et al.	

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/TS/	1	2006/084132	WO	A2	2006-08-10	Agencourt Bioscience Corp		☐

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	Examiner Name	T. E. Strzelecka
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/TS/	1	English translation of Notice of Reasons for Rejection mailed on November 13, 2014, for Japanese Patent Application No. 2013-546224, corresponding to International Patent Application Serial No. PCT/US2011/065098, with International Filing Date December 15, 2011 (8 pages)	☐
/TS/	2	Meyer et al., 2007, Targeted high-throughput sequencing of tagged nucleic acid samples, Nucleic Acids Research 35 (15):e97 (5 pages).	☐
/TS/	3	Smith et al., 2010, Highly-multiplexed barcode sequencing: an efficient method for parallel analysis of pooled samples, Nucleic Acids Research 38(13):e142 (8 pages).	☐

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Examiner Signature	/Teresa Strzelecka/	Date Considered	12/29/2014

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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013	02/28/2014	09/02/2014	12/29/2014					
	1	✓	✓	✓	✓					
	2	✓	✓	✓	✓					
	3	✓	✓	✓	✓					
	4	N	N	N	N					
	5	✓	✓	-	-					
	6	N	N	-	-					
	7	✓	✓	✓	✓					
	8	✓	✓	✓	✓					
	9	N	N	N	N					
	10	✓	✓	✓	✓					
	11	✓	✓	✓	✓					
	12	✓	✓	✓	✓					
	13	✓	✓	✓	✓					
	14	✓	✓	✓	✓					
	15	✓	✓	✓	✓					
	16	✓	✓	✓	✓					
	17	N	N	N	N					
	18	N	N	N	N					
	19	N	N	N	N					
	20	✓	✓	✓	✓					
	21	✓	✓	✓	✓					
	22	✓	✓	✓	✓					
	23	N	N	N	N					
	24	✓	✓	✓	✓					
	25	✓	✓	✓	✓					
	26	N	N	N	N					
	27	✓	✓	✓	✓					
	28	✓	✓	✓	✓					
	29	✓	✓	✓	✓					
	30	✓	✓	✓	✓					
	31	✓	✓	✓	✓					
	32	✓	✓	✓	✓					
	33	N	N	-	-					
	34	N	N	-	-					
	35	✓	✓	✓	✓					
	36	✓	✓	✓	✓					

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	**Art Unit**
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013	02/28/2014	09/02/2014	12/29/2014					
	37	N	N	N	N					
	38	✓	✓	✓	✓					
	39	✓	✓	-	-					
	40	N	N	-	-					
	41	✓	✓	✓	✓					
	42	N	N	N	N					
	43	N	N	N	N					
	44	N	N	N	N					
	45	N	N	N	N					
	46	N	N	N	N					

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
IDS (5/24/12; 8/6/12; 5/6/13)	6/7/13	/TS/
Inventor name search	6/7/13	/TS/
WEST	6/7/13, 6/10/13	/TS/
IDS (6/9/14, 7/1/14)	9/2/14	/TS/
WEST	9/2/14	/TS/
Inventor name search	9/2/14	/TS/
IDS (10/15/14; 12/22/14)	12/29/14	/TS/

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
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REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	13081660	Filing Date	2011-04-07	Docket Number (if applicable)	GSGE-001/01US 29258/14	Art Unit	1637
First Named Inventor	Gregory Porreca			Examiner Name	T. E. Strzelecka		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☒ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 50-0369

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
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Signature of Registered U.S. Patent Practitioner			
Signature	/Thomas C. Meyers/	Date (YYYY-MM-DD)	2015-04-06
Name	Thomas C. Meyers	Registration Number	36989

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal	
Application Number:	13081660
Filing Date:	07-Apr-2011
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Filer:	Thomas C. Meyers/Lauren Poon
Attorney Docket Number:	GSGE-001/01US 29258/14
Filed as Small Entity	
Filing Fees for Utility under 35 USC 111(a)	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
RCE- 2nd and Subsequent Request	2820	1	850	850
		Total in USD ($)		**850**

Electronic Acknowledgement Receipt	
EFS ID:	21974320
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Lauren Poon
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	06-APR-2015
Filing Date:	07-APR-2011
Time Stamp:	11:53:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$850
RAM confirmation Number	7458
Deposit Account	500369
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		GSGE-001-01US_Response-to-OA-010515.pdf	58665 8499ffde752848a424a2065389caeeb36a638dbf	yes	13

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment Submitted/Entered with Filing of CPA/RCE	1	1
	Claims	2	8
	Applicant Arguments/Remarks Made in an Amendment	9	13

Warnings:					
Information:					
2	Request for Continued Examination (RCE)	29258_14US-RequestforContinuedExamination.PDF	697727 4a163dc1e48c5dcfe473ed3fe930960825db72b3	no	3
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30938 d62077610eb4cb8f8c22ae38e2de9e70ddd292ae	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	787330		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Porreca et al.	ART UNIT:	1637
SERIAL NUMBER:	13/081,660	CONFIRMATION NO.:	4441
FILING DATE:	April 7, 2011	EXAMINER:	Strzelecka, Teresa E.
TITLE:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION		

FILED ELECTRONICALLY

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND REQUEST FOR CONTINUED EXAMINATION

This paper is in response to the final office action mailed January 5, 2015 from the United States Patent and Trademark Office. Applicants submit herewith a Request for Continued Examination (RCE), and pay the RCE fee. Applicants believe no other fee is due with this response, but nevertheless, if any fee is required, Applicants authorize the Commissioner to charge any required fees due to Deposit Account No. 50-0369.

Amendments to the claims begin on page 2.

Remarks begin on page 9.

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

1. (Currently Amended) A method for validating the integrity of a nucleic acid template in a multiplex sequencing reaction, the method comprising:

obtaining a template nucleic acid;

incorporating at least two of the same sequence identifiers into the template, wherein the sequence identifiers comprise four or more nucleotides;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the two incorporated sequence identifiers remain associated with the template, thereby validating the integrity of the nucleic acid template in a multiplex sequencing reaction.

2. (Original) The method according to claim 1, wherein a first identifier is incorporated into a 5' portion of the template and a second identifier is incorporated into a 3' portion of the template.

3. (Original) The method according to claim 2, wherein after incorporation of the identifiers, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

4. (Withdrawn) The method according to claim 1, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

5-6. (Canceled)

7. (Original) The method according to claim 1, wherein prior to sequencing, the template is attached to a substrate.

8. (Original) The method according to claim 2, wherein the template is directly attached to the substrate.

9. (Withdrawn) The method according to claim 2, wherein the template is indirectly attached to the substrate.

10. (Original) The method according to claim 7, further comprising, amplifying the template.

11. (Original) The method according to claim 1, wherein sequencing is sequencing by synthesis.

12. (Original) The method according to claim 11, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

13. (Original) The method according to claim 1, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

14. (Original) The method according to claim 13, wherein the detectably labeled nucleotide is an optically labeled nucleotide.

15. (Original) The method according to claim 14, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

16. (Original) The method according to claim 1, wherein the pair of identifiers are first and second barcode sequences that are attached to the template.

17. (Withdrawn) The method according to claim 16, wherein a first barcode sequence is attached to a 5' end of the template and a second barcode sequence is attached to a 3' end of the template.

18. (Withdrawn) The method according to claim 17, wherein the first and second barcode sequences are the same.

19. (Withdrawn) The method according to claim 17, wherein the first and second barcode sequences are different.

20. (Currently Amended) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a pair of the same barcode sequences to the template, wherein the barcodes comprise four or more nucleotides;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

21. (Previously presented) The method according to claim 20, wherein a first barcode sequence is incorporated into a 5' portion of the template and a second barcode sequence is incorporated into a 3' portion of the template.

22. (Original) The method according to claim 21, wherein after attaching of the pair of barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

23. (Withdrawn) The method according to claim 20, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

24. (Original) The method according to claim 20, wherein prior to sequencing, the template is attached to a substrate.

25. (Original) The method according to claim 24, wherein the template is directly attached to the substrate.

26. (Withdrawn) The method according to claim 24, wherein the template is indirectly attached to the substrate.

27. (Original) The method according to claim 24, further comprising, amplifying the template.

28. (Original) The method according to claim 20, wherein sequencing is sequencing by synthesis.

29. (Original) The method according to claim 28, wherein the sequencing by synthesis is single molecule sequencing by synthesis.

30. (Original) The method according to claim 20, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

31. (Previously presented) The method according to claim 30, wherein the labeled nucleotide is an optically labeled nucleotide.

32. (Original) The method according to claim 31, wherein the optically labeled nucleotide is a fluorescently labeled nucleotides.

33-34. (Canceled)

35. (Currently Amended) A method for analyzing a nucleic acid, the method comprising:

obtaining a nucleic acid template;

attaching a first barcode sequence to a 5' portion of the template and a second barcode sequence to a 3' portion of the template, wherein the first and second barcode sequences are the same, and wherein the barcode sequences comprise four or more nucleotides;

amplifying the template;

sequencing the template to generate sequence reads, and

analyzing the sequence reads to determine if the pair of attached barcode sequences remain associated with the template.

36. (Original) The method according to claim 35, wherein after attaching of the first and second barcode sequences, the template has the following configuration from 5' to 3': a first known sequence; a first barcode sequence; a second known sequence; a target polynucleotide sequence; a third known sequence; a second barcode sequence; and a fourth known sequence.

37. (Withdrawn) The method according to claim 35, wherein a first identifier is incorporated into a 5' end of the template and a second identifier is incorporated into a 3' end of the template.

38. (Original) The method according to claim 35, wherein prior to amplifying, the template is immobilized to a solid support.

39-40. (Canceled)

41. (Original) The method according to claim 35, wherein sequencing comprises:

hybridizing a primer to the template to form a template/primer duplex;

contacting the duplex with a polymerase enzyme in the presence of at least one detectably labeled nucleotide under conditions that permit the polymerase to add nucleotides to the primer in a template-dependent manner;

detecting a signal from the incorporated labeled nucleotide; and

sequentially repeating the contacting and detecting steps at least once, wherein sequential detection of incorporated labeled nucleotide determines the sequence of the nucleic acid.

42. (Withdrawn) A method for validating detection of an analyte, the method comprising detecting the presence of two or more identifiers that are uniquely associated with said analyte.

43. (Withdrawn) The method of claim 42, wherein said identifiers are nucleic acid sequence tags.

44. (Withdrawn) The method of claim 43, wherein said sequence tags are associated with a moiety that binds said analyte.

45. (Withdrawn) The method of claim 42, wherein said analyte is a protein.

46. (Withdrawn) The method of claim 45, wherein said identifiers are peptides.

Remarks

Claims 1-3, 7, 8, 10-16, 18, 20-22, 24, 25, 27-32, 35, 36, 38, and 41 remain pending in the application. Claims 1, 20, and 35 are amended. Support for the amendments can be found at page 12, lines 19-20, of the application as filed. No new matter is added.

Rejection under 35 U.S.C. §102(b)

The Office Action maintains the rejection of claims 1-3, 7-8, 10-12, 16, 20-22, 24-25, 27-29, 35-36, and 38 under 35 U.S.C. §102(b) over Parameswaran (Nucl. Acids. Res., 35:e130, pp. 1-9, 2007). Applicants submit that the amended claims are not anticipated by Parameswaran.

The legal standard for a novelty rejection is that the Patent Office must provide a single prior art reference that discloses each and every element of a claim (M.P.E.P. §2131). Claim 1 recites incorporating at least two of the same sequence identifiers into the template, wherein the sequence identifiers comprise four or more nucleotides. Likewise, claim 20 recites a pair of the same barcode sequences to the template, wherein the barcodes comprise four or more nucleotides. And claim 35 recites first and second barcode sequences that are the same, wherein the barcode sequences comprise four or more nucleotides. Parameswaran does not teach incorporating into a nucleic acid template two of the same sequence identifiers or barcodes of four or more nucleotides. Therefore, Parameswaran is missing that element of the claims.

Parameswaran reports a method of sequencing that involves attaching a forward barcode and a reverse barcode to a nucleic acid template, the forward and reverse barcodes having different designs from each other. According to Parameswaran, "[a]ll forward barcodes were of the type HIJJK LLMNO, while reverse barcodes were of the type HIJKK LMMNO. H, I, J, K and L, M, N, O are uniquely partnered with one of four nucleotides: A, C, G and T" (Parameswaran, page 4, col 2, lines 16-20). Parameswaran emphasizes the importance of that particular sequence design, stating: "barcode pairs were . . . designed, with any two forward or reverse barcodes differing in at least 4nt . . . . These serve as strong unique identifiers for each of

the forward and reverse barcode sets" (*Id*, lines 52-56). The forward barcode (HIJJK LLMNO) and the reverse barcode (HIJKK LMMNO), as required by Parameswaran, do not include any four-nucleotide sequence that is identical to each other. In fact, Parameswaran is silent on the element of incorporating two of the same barcodes into a template, wherein the barcodes comprise four nucleotides. Therefore, Parameswaran lacks that element of the independent claims.

Accordingly, Parameswaran does not anticipate any of the present claims. Applicants respectfully request withdrawal of the 35 U.S.C. §102(b) rejection.

Rejection under 35 U.S.C. §103(a)

The Office Action rejects claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38, and 41 under 35 U.S.C. §103(a) in view of the combination of Kawashima (U.S. patent application publication number 2005/0100900), Parameswaran, and Kircher et al. (Bioessays, 32:524-536, 2010). This rejection appears to be based on the disclosure in paragraph [00183] and FIGS. 13 and 15 of Kawashima. Applicants submit that the amended claims are not made obvious by any combination of Kawashima, Parameswaran, and Kircher.

In order to establish a prima facie case of obviousness, three basic criteria must be established by the Patent Office. First, there must be a suggestion in the cited art to modify a reference or to combine multiple references in a manner that would lead one of ordinary skill in the art to an applicant's claimed invention. Second, the cited references themselves must convey a reasonable expectation of success in arriving at the claimed invention based upon the proposed modification or combination. Finally, the teaching or suggestion to make the claimed invention must be found in the prior art and not in the applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). In addition to the requirements for making a combination, an obviousness rejection cannot be established in the first place unless each claim element is disclosed in the cited art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

The Examiner's rejection over the cited references relies on a claim interpretation that defines "sequence identifier" and "barcode" as a "sequence of two or more nucleotides." The amended claims, however, include the element of the barcodes or sequence identifiers comprising four or more nucleotides. Therefore, the Examiner's rejections based on that claim interpretation are moot. No combination of the cited references teaches or suggests the presently amended claims.

Kawashima reports a method of extending primers attached to a solid support. Kawashima reports that different tags can be used for different samples (Kawashima, paragraph [0183]). However, Kawashima does not disclose or suggest incorporating into a nucleic acid template two of the same sequence identifiers or barcodes comprising four or more nucleotides, as required by the present claims.

On page 7 of the Office Action, the Examiner states that a barcode can be defined as a sequence of two or more nucleotides and "[t]herefore Fig. 15 of Kawashima et al. clearly shows the same tag on both 5' and 3' ends of a target." However, FIG. 15 of Kawashima does not show any such thing. FIG. 15 merely shows something labeled "tag" incorporated into a sequence. But FIG. 15 is not described anywhere in the specification, and so it is entirely unknown what the "tags" indicated in that figure represent. There is no indication whatsoever that the "tags" comprise identical sequences of even two nucleotides. For that same reason, FIG. 15 of Kawashima does not teach four or more nucleotides, as required by the presently amended claims.

The Examiner also points to the sequence tags of FIG. 13 to allege that Kawashima teaches the use of identical tags. FIG. 13 shows tags having the following sequences: CATGTAC and CACG; and GTACATG and CGTG. Those tags do not include any identical four-nucleotide sequence, and therefore FIG. 13 also fails to disclose all of the elements of the present claims.

The description in FIGS. 13a-e is the only place in the specification of Kawashima where the composition of the tags is described, and that description is silent on tags having identical

sequences of four or more nucleotides. Therefore, Kawashima fails to disclose or suggest attaching two or more of the same tag to a sample, wherein the tag comprises four or more nucleotides.

None of the other cited references make up for what Kawashima lacks. As discussed above, Parameswaran is limited to reporting barcode sequences designed specifically so that they do not include any identical four-nucleotide sequence. Kircher is a review article limited to reporting different types of sequencing platforms. Kircher fails to disclose or suggest any type of identifier, let alone a method that involves incorporating at least two of the same sequence identifiers into a nucleic acid template.

Accordingly, the amended claims are not obvious in view of any combination of Kawashima, Parameswaran, and Kircher, because those references fail to disclose or suggest incorporating at least two of the same sequence identifiers into a nucleic acid template, wherein the sequence identifiers comprise four or more nucleotides.

Applicants respectfully request withdrawal of the 35 U.S.C. §103(a) rejection.

Summary

On the basis of the foregoing amendments and reasons, Applicants respectfully submit that the pending claims are in condition for allowance, which is respectfully requested.

If there are any questions regarding these remarks, the Examiner is invited and encouraged to contact Applicants' representative.

Dated: April 6, 2015

Respectfully submitted,

BROWN RUDNICK LLP

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111
Tel: (617) 856-8483
Fax: (617) 856-8201

/Thomas C. Meyers/

Thomas C. Meyers, Reg. No. 36,989
Attorney for Applicant
Email: tmeyers@brownrudnick.com

61898054

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/081,660	Filing Date 04/07/2011	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT **04/06/2015**

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 40	Minus	** 46	= 0	X $40 =	0
Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $210 =	0
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	0

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/DORIS BURNS/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

21710 7590 08/03/2015
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER
STRZELECKA, TERESA E

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 08/03/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

TITLE OF INVENTION: METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	11/03/2015

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 02/11)

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

21710 7590 08/03/2015
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

TITLE OF INVENTION: METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	11/03/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
STRZELECKA, TERESA E	1637	435-006100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

21710 7590 08/03/2015

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER	
STRZELECKA, TERESA E	

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 08/03/2015

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 13/081,660	Applicant(s) PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *RCe filed 4/6/15*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-4, 7-17,20-32,35-38 and 41*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has **T**HREE MON**T**HS FROM **T**HE "MAILING DA**T**E" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMEN**T** FOR **T**HE DEPOSI**T** OF BIOLOGICAL MA**T**ERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *7/14/15* .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

1. The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on April 6, 2015 has been entered.

3. Claims 1-4, 7-32, 35-38 and 41-46 were previously pending, with claims 4, 9, 17-19, 23, 26, 37 and 42-46 withdrawn from consideration. Applicant amended claims 1, 20 and 35.

4. Applicant's amendments overcame the previously presented rejections. No new references were found teaching or suggesting claims 1-3, 7, 8, 10-16, 20-22, 24, 25, 27-32, 35, 36, 38 and 41, therefore these claims are allowed. The previously withdrawn claims 4, 9, 17, 23, 26 and 37 are rejoined with the allowed claims.

EXAMINER'S AMENDMENT

5. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Thomas Meyers on July 14, 2015.

The application has been amended as follows:

Cancel claims 18, 19 and 42-46.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

TERESA E STRZELECKA
Primary Examiner
Art Unit 1637

/TERESA E STRZELECKA/
Primary Examiner, Art Unit 1637
July 22, 2015

Examiner-Initiated Interview Summary	Application No. 13/081,660	Applicant(s) PORRECA ET AL.	
	Examiner TERESA E. STRZELECKA	Art Unit 1637	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Teresa Strzelecka*. (3)_____.

(2) *Thomas Meyers*. (4)_____.

Date of Interview: *14 July 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *18,19 and 42-46*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Mr. Meyers was contacted regarding cancellation of previously withdrawn claims 18, 19 and 42-46. An independent claim 1, from which claims 18 and 19 depend via claims 16 and 17, is allowable, but the claims no longer limit claim 17, therefore would need to be cancelled. Claims 42-46 are drawn to a previously non-elected group, and would need to be canceled as well. Mr. Meyers agreed to cancellation of claims 18, 19 and 42-46 by examiner's amendment, therefore putting the claims in condition for allowance.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
IDS (5/24/12; 8/6/12; 5/6/13)	6/7/13	/TS/
Inventor name search	6/7/13	/TS/
WEST	6/7/13, 6/10/13	/TS/
IDS (6/9/14, 7/1/14)	9/2/14	/TS/
WEST	9/2/14	/TS/
Inventor name search	9/2/14	/TS/
IDS (10/15/14; 12/22/14)	12/29/14	/TS/

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
	C12Q1/6869 |C12Q2537/143 | C12Q2563/185	7/9/15	/TS/
	WEST	7/9/15	/TS/

WEST Search History for Application 13081660

Creation Date: 2015070912:57

Interference Searches

Query	DB	Op.	Plur.	Thes.	Date
(barcod $ 5 or tag $ 5 or index $ 5) near3 duplicat $ 5 and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	UPAD	ADJ	YES	ASSIGNEE	07-09-2015
((barcod $ 5 or tag $ 5 or index $ 5) near3 ("same" or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	UPAD	ADJ	YES	ASSIGNEE	07-09-2015

Prior Art Searches

Query	DB	Op.	Plur.	Thes.	Date
6403320.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
20090163366	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20080269068	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20100035243 or 20100297626 or 20100330619	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20070166705	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20090226975	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
20050100900	PGPB	ADJ	YES	ASSIGNEE	06-07-2013
7115400.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
7057026.pn.	USPT	ADJ	YES	ASSIGNEE	06-07-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parrallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6 or barcod$5) and sequencing	USPT	ADJ	YES	ASSIGNEE	06-10-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6 or barcod$5) and sequencing	USPT	ADJ	YES	ASSIGNEE	06-10-2013
(good start genetics.asn. or Capital Royalty Partners.asn. or Parallel Investment Opportunities.asn. or Bridge Bank.asn.) and (tag$6	PGPB	ADJ	YES	ASSIGNEE	06-10-2013

or barcod$5) and sequencing					
US-4683195-$.DID. OR US-4683202-$.DID. OR US-4988617-$.DID. OR US-5234809-$.DID. OR US-5242794-$.DID. OR US-5494810-$.DID. OR US-5583024-$.DID. OR US-5604097-$.DID.	USPT	ADJ	YES	ASSIGNEE	09-02-2014
US-5636400-$.DID. OR US-5674713-$.DID. OR US-5695934-$.DID. OR US-5700673-$.DID. OR US-5701256-$.DID. OR US-5830064-$.DID. OR US-5846719-$.DID. OR US-5863722-$.DID. OR US-5866337-$.DID. OR US-5869252-$.DID. OR US-5871921-$.DID.	USPT	ADJ	YES	ASSIGNEE	09-02-2014
US-5993611-$.DID. OR US-6100099-$.DID. OR US-6138077-$.DID. OR US-6150516-$.DID. OR US-6172214-$.DID. OR US-6172218-$.DID. OR US-6197508-$.DID. OR US-6210891-$.DID. OR US-6223128-$.DID. OR US-6235472-$.DID. OR US-6235475-$.DID.	USPT	ADJ	YES	ASSIGNEE	09-02-2014
US-6235501-$.DID. OR US-6258568-$.DID. OR US-6274320-$.DID. OR US-6306597-$.DID. OR US-6352828-$.DID. OR US-6361940-$.DID. OR US-6489105-$.DID. OR US-6558928-$.DID. OR US-6582938-$.DID. OR US-6585938-$.DID. OR US-6714874-$.DID.	USPT	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 (same or symmetric or idetical)	USPT	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 (same sequence or symmetric or identical)	USPT	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical)	USPT	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical)	USPT	ADJ	YES	ASSIGNEE	09-02-2014
((barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical)) and sequencing	USPT	ADJ	YES	ASSIGNEE	09-02-2014
((barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical)) same sequencing	USPT	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical) same librar$5	USPT	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical) same librar$5	PGPB	ADJ	YES	ASSIGNEE	09-02-2014
	PGPB	ADJ	YES	ASSIGNEE	09-02-2014

(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical) same librar$5					
(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical) same librar$5	PGPB	ADJ	YES	ASSIGNEE	09-02-2014
(barcod$5 or tag$5 or index$5) near3 ("same sequence" or symmetric or identical) same sequencing	PGPB	ADJ	YES	ASSIGNEE	09-02-2014
multiple near3 (barcod$5 or index$5) same sequencing	USPT	ADJ	YES	ASSIGNEE	09-02-2014
multiple near3 (barcod$5 or index$5) same sequencing	PGPB	ADJ	YES	ASSIGNEE	09-02-2014
multiple near3 (barcod$5 or index$5) same sequencing	DWPI	ADJ	YES	ASSIGNEE	09-02-2014
US-20080269068-A1.did.	PGPB	ADJ	YES	ASSIGNEE	09-02-2014
20080269068	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
13/081660.app.	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
((barcod$5 or tag$5 or index$5) near3 (same or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
((barcod$5 or tag$5 or index$5) near3 ("same" or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
(((barcod$5 or tag$5 or index$5) near3 ("same" or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)) and ("same" or symmetric or identical) near3 sequence	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
(((barcod$5 or tag$5 or index$5) near3 ("same" or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.) and ("same" or symmetric or identical) near3 sequence) and sequenc$8 near3 error	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
((barcod$5 or tag$5 or index$5) near3 ("same" or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	USPT	ADJ	YES	ASSIGNEE	07-09-2015
(((barcod$5 or tag$5 or index$5) near3 ("same" or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185	USPT	ADJ	YES	ASSIGNEE	07-09-2015

).CPC.)) and ("same" or symmetric or identical) near3 sequence and sequenc$8 near3 error					
((barcod$5 or tag$5 or index$5) near3 ("same" or symmetric or identical)) and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	DWPI	ADJ	YES	ASSIGNEE	07-09-2015
(barcod$5 or tag$5 or index$5) near3 duplicat$5 and sequencing and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
first near3(barcod$5 or tag$5 or index$5) and second near3(barcod$5 or tag$5 or index$5) and sequencing and first near3 ("same" or identical or symmetric) and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
first near3(barcod$5 or tag$5 or index$5) and second near3(barcod$5 or tag$5 or index$5) and sequencing and first near3 ("same" or identical or symmetric) near3 second and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	PGPB	ADJ	YES	ASSIGNEE	07-09-2015
first near3 (barcod$5 or tag$5 or index$5) and second near3(barcod$5 or tag$5 or index$5) and sequencing and first near3 ("same" or identical or symmetric) near3 second and ((C12Q1/6869 | C12Q2537/143 | C12Q2563/185).CPC.)	USPT	ADJ	YES	ASSIGNEE	07-09-2015

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	**Art Unit**
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013	02/28/2014	09/02/2014	12/29/2014	07/22/2015				
	1	✓	✓	✓	✓	=				
	2	✓	✓	✓	✓	=				
	3	✓	✓	✓	✓	=				
	4	N	N	N	N	=				
	5	✓	✓	-	-	-				
	6	N	N	-	-	-				
	7	✓	✓	✓	✓	=				
	8	✓	✓	✓	✓	=				
	9	N	N	N	N	=				
	10	✓	✓	✓	✓	=				
	11	✓	✓	✓	✓	=				
	12	✓	✓	✓	✓	=				
	13	✓	✓	✓	✓	=				
	14	✓	✓	✓	✓	=				
	15	✓	✓	✓	✓	=				
	16	✓	✓	✓	✓	=				
	17	N	N	N	N	=				
	18	N	N	N	N	-				
	19	N	N	N	N	-				
	20	✓	✓	✓	✓	=				
	21	✓	✓	✓	✓	=				
	22	✓	✓	✓	✓	=				
	23	N	N	N	N	=				
	24	✓	✓	✓	✓	=				
	25	✓	✓	✓	✓	=				
	26	N	N	N	N	=				
	27	✓	✓	✓	✓	=				
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	29	✓	✓	✓	✓	=				
	30	✓	✓	✓	✓	=				
	31	✓	✓	✓	✓	=				
	32	✓	✓	✓	✓	=				
	33	N	N	-	-	-				
	34	N	N	-	-	-				
	35	✓	✓	✓	✓	=				
	36	✓	✓	✓	✓	=				

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	Art Unit
	TERESA E STRZELECKA	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/10/2013	02/28/2014	09/02/2014	12/29/2014	07/22/2015				
	37	N	N	N	N	=				
	38	✓	✓	✓	✓	=				
	39	✓	✓	-	-	-				
	40	N	N	-	-	-				
	41	✓	✓	✓	✓	=				
	42	N	N	N	N	-				
	43	N	N	N	N	-				
	44	N	N	N	N	-				
	45	N	N	N	N	-				
	46	N	N	N	N	-				

PALM INTRANET

Day : Thursday

Date: 7/9/2015
Time: 10:23:39

Inventor Name Search

Enter the **first few letters** of the Inventor's Last Name.
Additionally, enter the **first few letters** of the Inventor's First name.

Last Name	First Name	
Porreca	Gregory	Search

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PALM INTRANET

Day : Thursday

Date: 7/9/2015
Time: 10:23:39

Inventor Name Search

Enter the **first few letters** of the Inventor's Last Name.
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Last Name	**First Name**	
Umbarger	Mark	Search

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PALM INTRANET

Day : Thursday

Date: 7/9/2015
Time: 10:23:39

Inventor Name Search

Enter the **first few letters** of the Inventor's Last Name.
Additionally, enter the **first few letters** of the Inventor's First name.

Last Name	**First Name**	
Church	George	Search

To Go BACK Use BACK Button on Your BROWSER Tool Bar
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Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	**Art Unit**
	TERESA E STRZELECKA	1637

CPC						
Symbol					**Type**	**Version**
C12Q		1	/	6869	F	2013-01-01
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CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
C12Q		1	/	6869	I	1	1	2013-01-01
C12Q		2537	/	143	A	1	2	2013-01-01
C12Q		2563	/	185	A	1	3	2013-01-01

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	33	
/TERESA E STRZELECKA/ Primary Examiner.Art Unit 1637	7/22/15	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	**Art Unit**
	TERESA E STRZELECKA	1637

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION									
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED				
		C	1	2	P	19 / 34 (2006.01.01)	C	0	7	H	21 / 02 (2006.01.01)
CROSS REFERENCE(S)							C	0	7	H	21 / 04 (2006.01.01)
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)										

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	33	
/TERESA E STRZELECKA/ Primary Examiner.Art Unit 1637	7/22/15	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13081660	PORRECA ET AL.
	Examiner	**Art Unit**
	TERESA E STRZELECKA	1637

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 33	
/TERESA E STRZELECKA/ Primary Examiner.Art Unit 1637 (Primary Examiner)	7/22/15 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure None

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

21710 7590 08/03/2015
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	4441

TITLE OF INVENTION: METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	11/03/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
STRZELECKA, TERESA E	1637	435-006100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Thomas C. Meyers
2 Brown Rudnick LLP
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Good Start Genetics, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Cambridge, MA

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 50-0369 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ☐ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Thomas C. Meyers/ Date September 15, 2015

Typed or printed name Thomas C. Meyers Registration No. 36989

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Electronic Patent Application Fee Transmittal				
Application Number:	13081660			
Filing Date:	07-Apr-2011			
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION			
First Named Inventor/Applicant Name:	Gregory Porreca			
Filer:	Thomas C. Meyers/Lauren Poon			
Attorney Docket Number:	GSGE-001/01US 29258/14			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	2501	1	480	480

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**480**

Electronic Acknowledgement Receipt	
EFS ID:	23491252
Application Number:	13081660
International Application Number:	
Confirmation Number:	4441
Title of Invention:	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
First Named Inventor/Applicant Name:	Gregory Porreca
Customer Number:	21710
Filer:	Thomas C. Meyers/Lauren Poon
Filer Authorized By:	Thomas C. Meyers
Attorney Docket Number:	GSGE-001/01US 29258/14
Receipt Date:	15-SEP-2015
Filing Date:	07-APR-2011
Time Stamp:	15:47:08
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$480
RAM confirmation Number	2277
Deposit Account	500369
Authorized User	MEYERS, THOMAS C.
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	GSGE-001-01US_IssueFee.pdf	96350 c9fe89045d2101d9e91a1eaa637245f7d89fdcd2	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30812 f7ac4e89b6d62b649afa540326684052190 49e4b	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	127162		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	13081660
Filing Date	2011-04-07
First Named Inventor	Gregory Porreca
Art Unit	1637
Examiner Name	T. E. Strzelecka
Attorney Docket Number	GSGE-001/01US

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /TS/

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	4683195		1987-07-28	Mullis et al.	
	2	4683202		1987-07-28	Mullis	
	3	4988617		1991-01-29	Landegren et al.	
Change(s) applied to document, /S.D./ 8/14/2015	4	5234809		1993-08-10	~~Akzo N.V.~~ Boom, et al.	
	5	5242794		1993-09-07	Whiteley et al.	
	6	5494810		1996-02-27	Barany et al.	
	7	5583024		1996-12-10	McElroy, deceased et al.	
	8	5604097		1997-02-18	Brenner	

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/081,660	10/20/2015	9163281	GSGE-001/01US 29258/14	4441

21710 7590 09/30/2015

BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 328 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Gregory Porreca, Cambridge, MA;
Mark Umbarger, Brookline, MA;
George Church, Brookline, MA;

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY.DOCKET NO./TITLE	REQUEST ID
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14	67503

Acknowledgement of Loss of Entitlement to Entity Status Discount

The entity status change request below filed through Private PAIR on 07/11/2018 has been accepted.

CERTIFICATIONS:

Change of Entity Status:

X Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be notification of loss of entitlement to small or micro entity status, as applicable.

This portion must be completed by the signatory or signatories making the entity status change in accordance with 37 CFR 1.4(d)(4).

Signature:	/Thomas C. Meyers/
Name:	Thomas C. Meyers
Registration Number:	36989

PTO/SB/81A (12-08)
Approved for use through 03/31/2021. OMB 0651-0035
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PATENT - POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Patent Number	9,163,281
Issue Date	October 20, 2015
First Named Inventor	Gregory PORRECA
Title	METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION
Attorney Docket No.	MLBS-001/001US 344251-2001

I hereby revoke all previous powers of attorney given in the above-identified patent.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the Customer Number identified in the box at right as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith: 58249

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number	Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified patent to:

☒ The address associated with the above-identified Customer Number.

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☐ The address associated with the Customer Number identified in the box at right:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☐ Inventor, having ownership of the patent.

OR

☒ Patent owner.

Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____ .

SIGNATURE of Inventor or Patent Owner

Signature	/ Gregory Porreca /	Date	03-Jan-2023
Name	Gregory Porreca	Telephone	978-254-7799
Title and Company	Molecular Loop Biosciences, Inc. President and CEO		

NOTE: Signatures of all the inventors or patent owners of the entire interest or their representative(s) are required. If more than one signature is required, submit multiple forms, check the box below, and identify the total number of forms submitted in the blank below.

☐ A total of 1 forms are submitted.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
13/081,660	**02/06/2023 04:05:16 PM ET**	**GSGE-001/01US 29258/14**

Title of Invention

METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	9163281
CONFIRMATION #	4441	FILED BY	Johanna Vecchia
PATENT CENTER #	61551434	FILING DATE	04/07/2011
CUSTOMER #	21710	FIRST NAMED INVENTOR	Gregory Porreca
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Edith Cheng

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
POA_MLBS_001_001US.pdf	1	Power of Attorney	89 KB
Statement_373c_MLBS_001_001US.pdf	1	Assignee showing of ownership per 37 CFR 3.73	77 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
POA_MLBS_001_001US.pdf	1AB920B69DBC637879CCC4E5DA6BFB960E444B5C210CF117341A470F7A80E86E02C3172C7DA99D82D7CCAAFFB8B42748D7435B94743B7A57D69B57F200918DDA

Statement_373c_MLBS_001_001US.pdf	75FC0A61898A55C062233F7EE603DD787332D7A6227C1609436CA1CD803598119BF861C42ECE2E8E002EBECBA1087456AEACCD278EF6CD8980041161FD77688C

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

MLBS-001/001US

PTO/SB/96 (11-18)
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STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Molecular Loop Biosciences, Inc.

Application No./Patent No.: 9,163,281 Filed/Issue Date: October 20, 2015

Titled: METHODS FOR MAINTAINING THE INTEGRITY AND IDENTIFICATION OF A NUCLEIC ACID TEMPLATE IN A MULTIPLEX SEQUENCING REACTION

Molecular Loop Biosciences, Inc. (Name of Assignee) , a corporation (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. [X] the assignee of the entire right, title, and interest in;
2. [ ] an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or
3. [ ] the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made) the patent application/patent identified above, by virtue of either:

A. [ ] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or a copy* is attached.

OR

B. [X] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Gregory PORRECA, Mark UMBARGER, George CHURCH To: GOOD START GENETICS, INC.
The document was recorded in the United States Patent and Trademark Office at
Reel 027200 , Frame 0369 , or a copy* is attached.
2. From: GOOD START GENETICS, INC. To: MOLECULAR LOOP BIOSOLUTIONS, LLC
The document was recorded in the United States Patent and Trademark Office at
Reel 047459 , Frame 0461 , or a copy* is attached.
3. From: MOLECULAR LOOP BIOSOLUTIONS, LLC To: MOLECULAR LOOP BIOSCIENCES, INC.
The document was recorded in the United States Patent and Trademark Office at
Reel 062066 , Frame 0759 , or a copy* thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet(s).

[X] *As required by 37 CFR 3.73(b)(1)(i), if a copy/copies is/are attached, the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Edith Hang Yu Cheng/ — Signature
February 6, 2023 — Date

Edith Hang Yu Cheng — Printed or Typed Name
Limited Recognition No. L1258 — Title or Registration Number

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/081,660	04/07/2011	Gregory Porreca	MLBS-001US 344251-2001

CONFIRMATION NO. 4441

58249
COOLEY LLP
ATTN: IP Docketing Department
1299 Pennsylvania Avenue, NW
Suite 700
Washington, DC 20004

POA ACCEPTANCE LETTER

OC000000137352995

Date Mailed: 02/08/2023

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 02/06/2023.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/nrhayden/

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/081,660	04/07/2011	Gregory Porreca	GSGE-001/01US 29258/14

CONFIRMATION NO. 4441

21710
BROWN RUDNICK LLP
ONE FINANCIAL CENTER
BOSTON, MA 02111

POWER OF ATTORNEY NOTICE

OC000000137352971

Date Mailed: 02/08/2023

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 02/06/2023.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/nrhayden/