



Illumina v. Molecular Loop

IPR2024-01419

Petitioner Illumina's Demonstrative Exhibits

January 29, 2026

Petitioner Illumina, Inc., Ex. 1070
Illumina Inc. v. Molecular Loop Biosciences, Inc.
IPR2024-01419

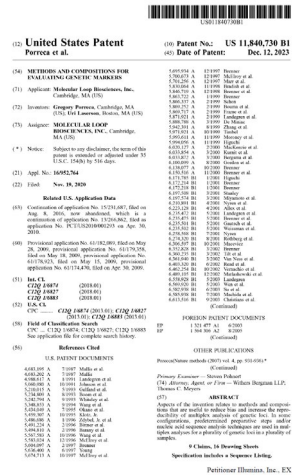
Grounds

		Ground	Claims	Basis	References
Chee	{	1	1-4, 9	§102	Chee
		2	1-4, 7, 9	§103	Chee
		3	5-6, 8	§103	Chee + Gahan
McCloskey	{	4	1, 4-6, 9	§103	Bignell + McCloskey
		5	2-3	§103	Bignell + McCloskey + Bentley
		6	6, 8	§103	Bignell + McCloskey + Gahan
		7	7	§103	Bignell + McCloskey + Chiang

Disputed Issues

- Grounds 4-7: McCloskey + Bignell
 - Whether McCloskey teaches the “collapsing” step of claim 1
 - Whether there is motivation to combine McCloskey and Bignell
- Grounds 1-3: Chee
 - Whether Chee teaches the “collapsing” step of claim 1
 - Whether Chee teaches the “consensus base call” of claim 2
 - Whether there is motivation to combine Chee with Gahan for claims 5, 6, and 8
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'730 Claim 1

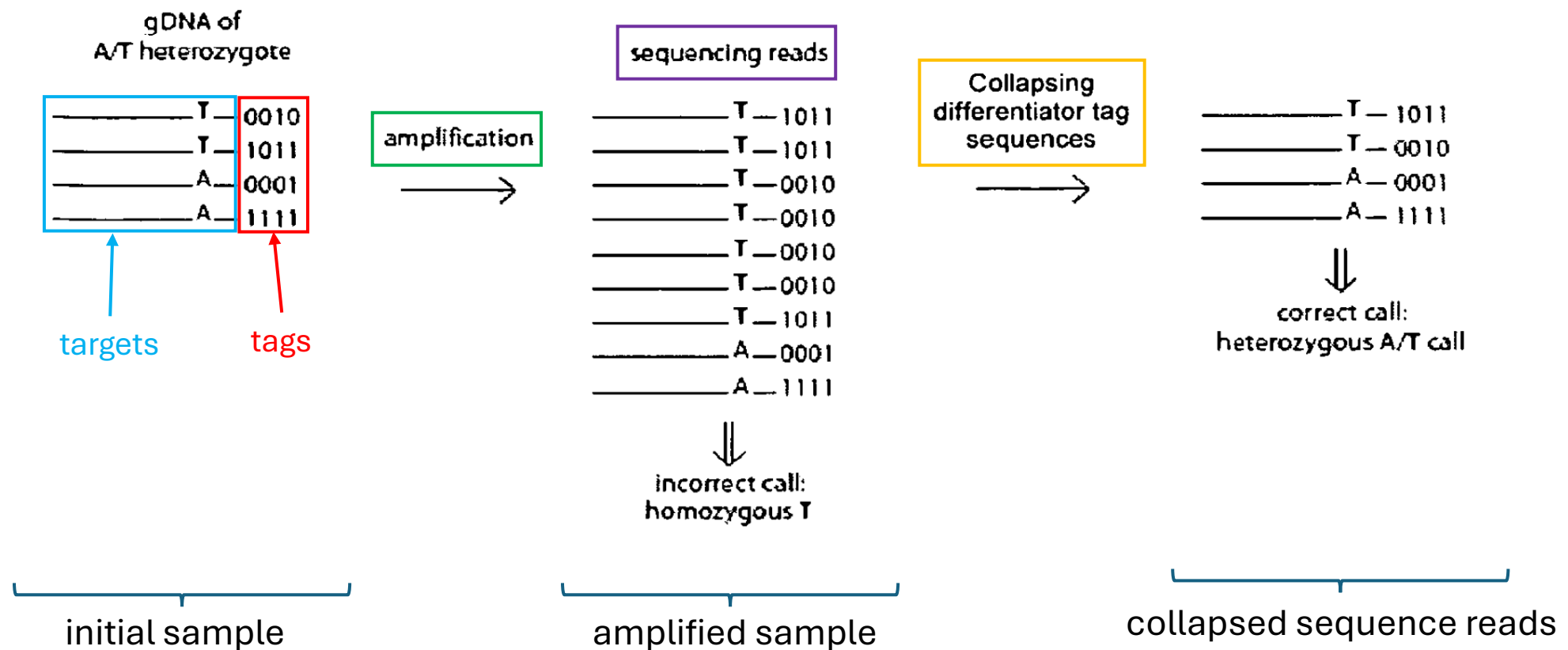


1. A method for correcting for errors or bias introduced during nucleic acid analysis workflow, the method comprising the steps of:

- obtaining a biological sample comprising a plurality of target nucleic acid molecules from more than one locus of origin;
- introducing a set of differentiator tags, wherein members of said set of differentiator tags are associated with members of said plurality, such that one or more of said loci of origin are associated with more than one differentiator tag;
- amplifying each of the plurality of tagged target nucleic acid molecules to generate amplicons;
- sequencing the amplicons obtained in said amplifying step to obtain sequence reads of each of the amplicons, wherein each of the sequence reads comprises a target nucleic acid molecule sequence and a differentiator tag sequence; and
- correcting for error or bias introduced during said workflow by **collapsing target:differentiator tag combinations observed more than once into a single count.**

'730 Figure 5

FIG. 5



Molecular Loop: Purpose of Collapsing Is to Correct for Skewing

Patent Owner Response:

Case IP2024-01419
U.S. Patent No. 11,840,730

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA INC.,
Petitioner

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner

Case IP2024-01419

U.S. Patent No. 11,840,730

PATENT OWNER'S RESPONSE

Mail Stop Patent Board
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

The specification of the '730 Patent also explains that **collapsing corrects for skewing**, an issue related to over- or under-amplification

PO Resp, 43

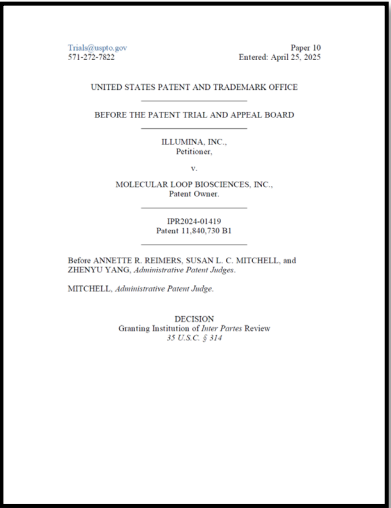
[C]ollapsing serves a different purpose: to **remove redundant sequence reads** prior to counting.

PO Resp, 46

[R]edundant amplicons with the same unique differentiator tag:target combinations **are removed to create collapsed sequence reads**, which resolves issues such as **over- and under-amplification**.

PO Resp, 10

The Board’s Constructions



Institution
Decision

Term	Construction
“set of differentiator tags” “set of unique differentiator tags”	set of prepared tags where each tag includes a unique sequence as compared to other tags
“locus of origin”	the location in a genome where a nucleic acid came from

Inst. Dec., 6-8

Disputed Issues

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Bignell + McCloskey

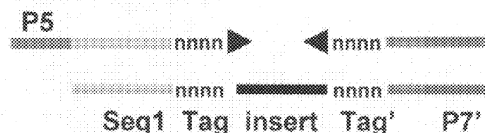
Bignell

United States Patent
Bignell et al.
Patent No.: US 8,853,192 B2
Date of Patent: Nov. 5, 2012

The invention relates to **methods for indexing samples during the sequencing** of polynucleotide templates, resulting in **the attachment of tags specific to the source of each nucleic acid sample** such that after a sequencing run, both the source and sequence of each polynucleotide can be determined.

EX1013, 1:24-28; Pet., 45

Amplification



EX1013, Fig. 3; Pet., 48-49

McCloskey

United States
Patent Application Publication
McCloskey et al.
Pub. No.: US 2007/0026640 A1
Pub. Date: Jun. 25, 2007

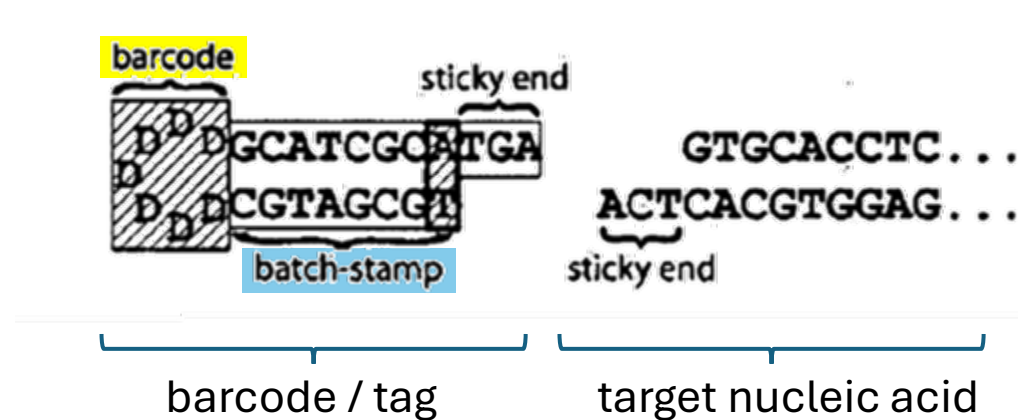
In the practice of the present invention **nucleic acid molecules** (e.g., genomic DNA fragments) **are labeled with distinct sequence tags prior to PCR amplification**. These sequence tags authenticate a nucleic acid sequence with information, such as... the sample identity.... **Barcoding permits**, for example, quantification of the relative abundance of genomic methylation patterns or polymorphic sequences by **correcting for skewing that can arise from PCR amplification** or the cloning of the products.

EX1012, [0005]; Pet., 47, 51

The **barcoding** method thus provides **a definitive solution** to the problem identified previously..., in which **multiple amplified sequences are derived from a single original molecule**.

EX1012, [0106]; Pet., 51

McCloskey



“a **random barcode**... can **uniquely identify a target nucleic acid** in an experiment.”

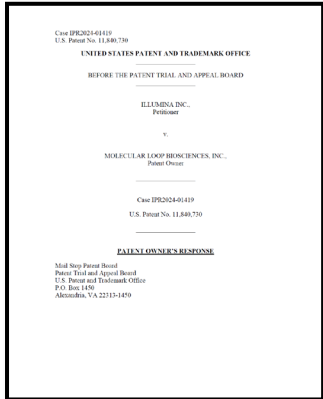
“a batch-stamp... may identify a sample, a patient, and/or an analysis date.”



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ML's Own Expert Admits McCloskey's Barcodes Correct Amplification Bias



Patent Owner Response:

The specification of the '730 Patent also explains that **collapsing corrects for skewing**, an issue related to over- or under-amplification

PO Resp, 43

[D]uring amplification, certain nucleic acid molecules from the original sample could be over- or under-amplified (a phenomenon known as **amplification bias**), resulting in a **skewed number of amplicons** attributable to a single original copy of a sequence.

PO Resp, 1



Dr. Puglisi
ML's Expert

Q **McCloskey's barcodes are used to correct for amplification bias?**

[A] : That is in the -- **that is stated as one of the applications in the specification**, but it is not what is claimed. Okay?

Q By "not what is claimed," you're saying, not what is claimed in McCloskey?

A In McCloskey's claims, yes....

So [s]he's definitely barcoding. There's no question about it. [She]'s using the barcodes as a way to address samples and combine them. **[She]'s using barcode -- claims to use barcodes as a way to correct for amplification bias. That, there's no doubt about.**

EX1066, 85:21-86:13; Reply, 4

McCloskey Discloses “Collapsing... Into a Single Count”

'730 Patent, Claim 1:

correcting for error or bias introduced during said workflow by **collapsing** target:differentiator tag combinations observed more than once **into a single count**.

McCloskey:

[G]enomic DNA fragments were labeled with... barcodes and “batch-stamps” prior to PCR amplification... **allowing us to identify... redundant sequences** and to quantify accurately the proportion of cells carrying a particular sequence variant by **counting only distinctly tagged sequences**.

EX1012, [0093]; Pet., 56-57; Reply, 4-5

A **redundant sequence** was also recovered, meaning that a MLM1-positive sequence **with an already seen barcode** was recovered. **One of these two sequences was counted** as a valid sequence.

EX1012, [0063]; Pet., 46, 54; EX1005, ¶¶278, 293

McCloskey Collapses Target:Tag Combinations Observed More than Once Into a Single Count

'730 Patent, Claim 1:

correcting for error or bias introduced during said workflow by collapsing **target:differentiator tag combinations observed more than once** into a single count.

McCloskey:

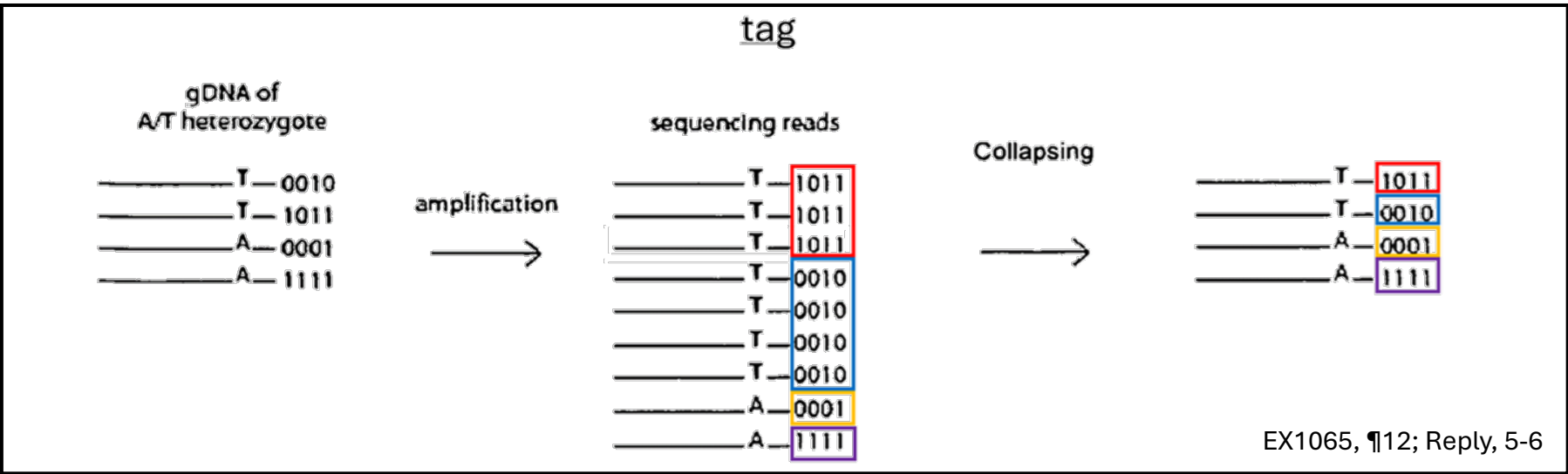
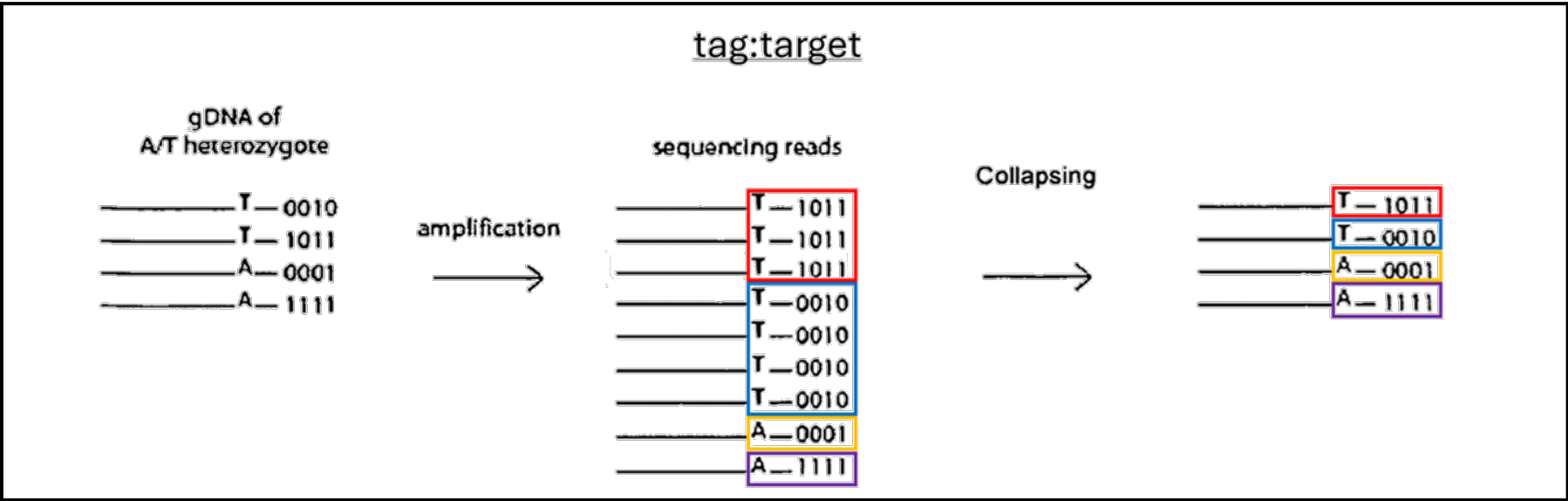
“a **random barcode**... can **uniquely identify a target nucleic acid** in an experiment.”

EX1012, [0021]; Pet., 54

Each of McCloskey's Barcodes Is Uniquely Associated with a Single Target Nucleic Acid in the Initial Sample



Dr. Gabriel
Illumina's Expert



EX1065, ¶12; Reply, 5-6

Claim 1's "Collapsing..." Encompasses Claim 2, Which Requires "Collapsing All Sequence Reads Comprising a Same Tag"

1. A method for correcting for errors or bias... comprising the steps of:

* * *

correcting for error or bias introduced during said workflow by **collapsing target:differentiator tag combinations observed more than once into a single count**.

2. The method of claim 1, wherein **said correcting step** further comprises **collapsing all sequence reads comprising a same differentiator tag into a single read** before determining a consensus base call for the target nucleic acid molecule sequences.

Reply, 4-5

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A POSA Would Have Recognized Bignell's Use of Several Amplification Steps Could Cause Bias



Dr. Gabriel
Illumina's Expert

251. **Bignell's** method includes **a number of amplification steps**. As I explained above, a person of ordinary skill in the art **would have known that the amplification process typically introduced bias or errors**.... Such bias could come from making more copies of some nucleic acids than others... **McCloskey teaches that its method of adding unique barcodes to each nucleic acid molecule provides a way to identify and correct for errors and/or bias introduced by amplification**... A person of ordinary skill in the art would have therefore recognized that incorporating McCloskey's unique barcodes with Bignell's method of Next-Gen sequencing would have provided a solution to errors and/or bias caused by the amplification steps.

EX1005, ¶1250; Pet., 47-48

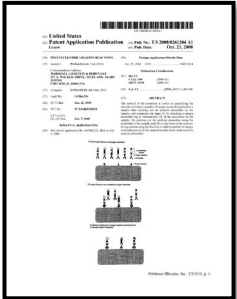
Skewing Caused by Amplification Was a Known Problem



Arcinas et al.:

The basic types of PCR artifacts have been shown in controlled laboratory studies and can be divided into two categories: those resulting in sequence artifacts (PCR errors), and those **skewing the distribution of PCR products due to unequal amplification (PCR bias)** or cloning efficiency.

EX1025, 8966; Pet., 47



Lexow:

For example, **in an amplification process one target molecule in a sample may be copied more times than another target molecule**, thereby making it difficult to measure the absolute and relative amounts of the different target molecules that were present in the original sample.

EX1018, [0002]; Pet., 47



Dr. Puglisi
ML's Expert

Q If the goal is to reduce skewing, there is no difference if you collapse tags alone or tags plus target in McCloskey's system?

[A]: Again, **the idea of correcting for skewing** in terms of the population that you're sequencing, okay, **was known in the art**. I mean, **that's... one of the reasons people introduced barcodes** back in the day, long **before even McCloskey's patent**.

EX1066, 79:17-80:11; Reply, 1

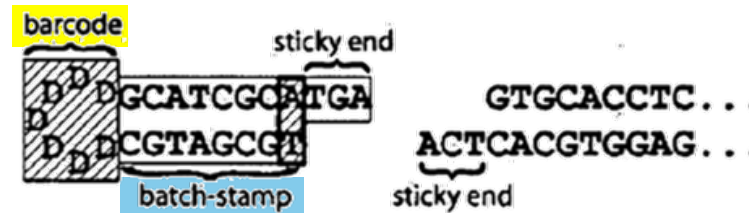
McCloskey Uses Both Barcodes and Sample Identifiers

Bignell

Such **tags are used to label target molecules in a sample** so that after an amplification reaction, each original molecule in the sample has a unique tag. **The tags can then be used to sort the sample onto beads...** (**Brenner** et al....). The problem with enabling the method for individual samples rather than individual molecules is that the tags are synthesised in a combinatorial manner, meaning that all 16,777,216 different sequences are obtained in a single mixture in the same tube. **Whilst this is ideal for treating one sample such that each individual molecule in the sample carries a different tag, it does not permit attachment of the same tag to every molecule in the sample.**

EX1013, 3:16-35; Reply, 8-9; PO Resp, 58

McCloskey



EX1012, Fig. 3; Pet., 7; EX1005, ¶182

“a **random barcode**... can **uniquely identify a target nucleic acid** in an experiment.”

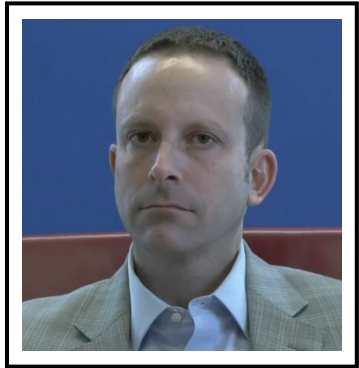
EX1012, [0021]; Pet., 54

“a **batch-stamp**... **may identify a sample**, a patient, and/or an analysis date.”

EX1012, [0027]; Pet., 49

Porreca's March 6, 2009 Email & Draft Application

March 6, 2009 Porreca Email



Dr. Porreca
Named Inventor

Please find drafts of our three provisional patent applications attached. Below is also a list of papers and patents associated with the drafts.

Aside from next steps working with you to refine the document and add information where needed, we'd like to discuss several topics on Wednesday:

1) have we defined the inventions broadly enough (or, is it possible to increase breadth further)?

2) how much overlap do you see in the "Bias-detecting barcodes" application with the patent reference called out in Note 1 on page 1 (McCloskey et al.)

3) how clear are the figures?

McCloskey et al., US Patent Application Publication number: US 2007/0020640 A1

Miner et al. 2004 Nucl. Acids Res. 32:e135

McCloskey et al. 2007 Biochemical Genetics 45:1-6

Olsen 2007 Nature Meth 4:891

EX2021, 1; EX2005, ¶17; PO Resp, 22, 24

March 6, 2009 Draft Application

[NOTE1: There is some prior art associated with random barcodes, but **I'm not sure if it anticipates the applications we are interested in**. See, mainly, USPTO Publication Number US 2007/0020640 A1 (**McCloskey** et al.).]

EX2006, 2

Cost Would Not Have Dissuaded a POSA from Making the Combination



Dr. Gabriel
Illumina's Expert

20... Synthesizing **random unique oligo barcodes would not have been a significant expense**, as was known in the art. **McCloskey describes several techniques for synthesizing oligos that were within the skill of those in the art.** EX1012(McCloskey), [0021]. I have extensive experience in managing the costs of sequencing and addressing trade-offs between cost and quality based on the goals of an experiment, as I run the largest sequencing lab in the United States, and have done so for the past 13 years. In my experience, **adding random barcodes to the sequence reads would not have significantly increased the cost of next-generation sequencing**, and to the extent that some additional cost is incurred in use cases where high accuracy is required, the added cost is warranted by the improved analytic accuracy. In any event, it would still be significantly less expensive to use McCloskey's unique barcodes with next-generation sequencing than with Sanger sequencing.

EX1065, ¶120; Reply, 9

Chee Used Unique Identifiers to Solve the Same Problem with Amplification Bias

Chee

In addition, the methods of the invention can **identify technical bias in, e.g., amplification**, as the unique identifier will be amplified with the ROI of each construct and thus **the number of starting molecules can be inferred by association of each initial ROI present in a sample with its unique identifier**.

EX1010, 10:41-46; Pet., 31, 50

Q So ignoring whether the source is natural or synthetic DNA, you agree that **Chee discloses correcting for amplification bias**, correct? ...

[A]: He -- what Chee is proposing is a method -- obviously, he needs to show that that method works, but **by just reading the unique identifiers, he is proposing that that would allow him to correct for amplification bias**.

EX1066, 122:12-20; Reply, 13



Dr. Puglisi
ML's Expert

McCloskey Leaves Selection of a Sequencing Technique to a POSA

McCloskey:

Generally, the amplification products may be analyzed by gel electrophoresis, and further cloning and sequencing appropriately sized products. **Methods and conditions for analyzing nucleic acid molecules by gel electrophoresis, cloning, and sequencing are standard in the art.**

EX1012, [0034]; Pet., 47



Dr. Gabriel
Illumina's Expert

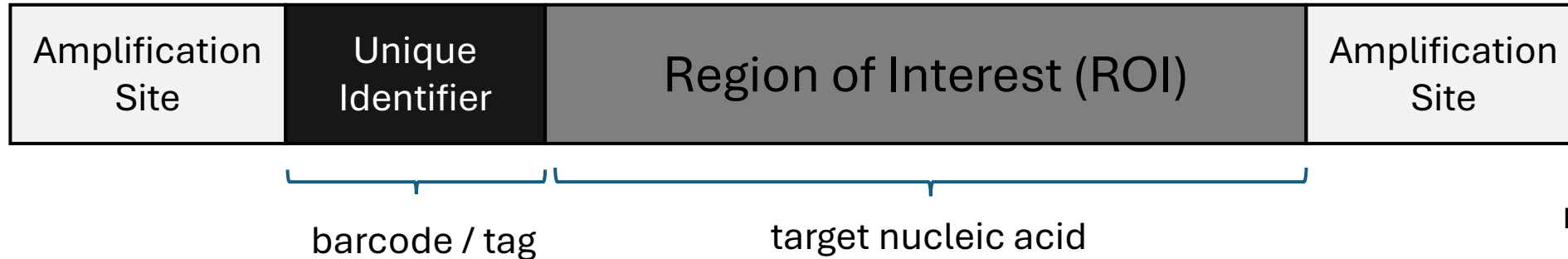
250. A person of ordinary skill in the art would have been motivated to **combine McCloskey specifically with a Next-Gen sequencing technique**, such as the ones described in Bignell. As I explained above, by early 2009, Next-Gen sequencing technologies were widely adopted because they provided sequencing methods which were both cost-effective and highly efficient... Next-Gen sequencing technologies have been praised **for “undeniably reinvigorating this field” and creating “the incentive for developing entirely new strategies for DNA sequencing.”**... It made sequencing more affordable and available for all types of experiments and analyses. Thus, a person of ordinary skill in the art **would have been motivated to implement a Next-Gen sequencing technique, such as the technique described by Bignell, with McCloskey's method.**

EX1005, ¶1250; Pet., 47

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Chee's Constructs



EX1010, Fig. 1; Pet., 21

Chee

The present invention provides a novel approach to ensuring sequence fidelity and/or quantifying the percentage of individual ROI sequences within a sample by creating nucleic acid constructs and selection methods for identifying particular ROIs in the constructs. The constructs of the invention have **a unique identifier** associated with the initial construct, thus giving the ability to differentiate between constructs having substantially the same ROI.

EX1010, 9:5-12; Pet., 32

In essence, the **'unique'** (i.e. unique for a construct comprising a particular ROI) **identifiers are associated with individual molecules in the starting sample**. Therefore, any **amplification products** of these initial individual molecules bearing the unique identifier are assumed to be identical by descent.

EX1010, 5:27-36; Pet., 28

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Chee Corrects Bias to Allow Accurate Counting of the Number of Molecules in the Starting Sample

Chee

In addition, the methods of the invention can **identify technical bias in, e.g., amplification**, as the unique identifier will be amplified with the ROI of each construct and thus **the number of starting molecules can be inferred** by association of each initial ROI present in a sample with its unique identifier.

EX1010, 10:41-46; Pet., 31

Improved Quantification of ROIs

Methods to determine the relative amounts of nucleic acids from a sample often require amplification methods in order to have enough material to carry out the desired testing.... By creating constructs comprising the nucleic acids directly isolated from a sample, bias due to subsequent manipulation of the molecules can be identified by determining the amounts of constructs with unique identifiers. Thus, if a nucleic acid is either over-represented or under-represented due to **amplification bias**, the number of molecules comprising unique identifiers will also be **over-represented or under-represented**, and **the quantification of the molecules can be corrected to reflect this manner of bias**.

EX1010, 23:50-64; Pet., 31

Chee Discloses How to Collapse Redundant Sequences Into a Single Count



Dr. Gabriel
Illumina's Expert

173. Chee explains that the unique identifiers allow it to determine **how many of each molecule was present in the initial sample**... Chee uses the unique identifiers to determine that two sequenced constructs are “identical by descent,” by which Chee means they are copies of (or descended from) the same construct from the original sample... This use of unique identifiers allows Chee to “correct” the “quantification of the molecules” to fix the bias... **By correcting the quantification**, Chee means that where it reads **multiple sequences with the same unique identifier, it only counts those as one instance of the ROI** (not multiple instances). **This “collapses” multiple reads into one count.**

EX1005, ¶173; Pet., 31-32

24... Chee says that uses of its unique identifiers allows “quantification of the molecules [to] be corrected to reflect [amplification] bias.” EX1010(Chee), 23:59-64; id., 9:5-9, 9:18-21. Quantification in this context means counting the number of molecules in the starting sample. **A POSA would have known how to count the distinct unique identifiers**, and how to use only one copy of a construct with a particular unique identifier to quantify the molecules in the starting sample **based on Chee's disclosure.**

EX1065, ¶124; Reply, 13

Disputed Issues

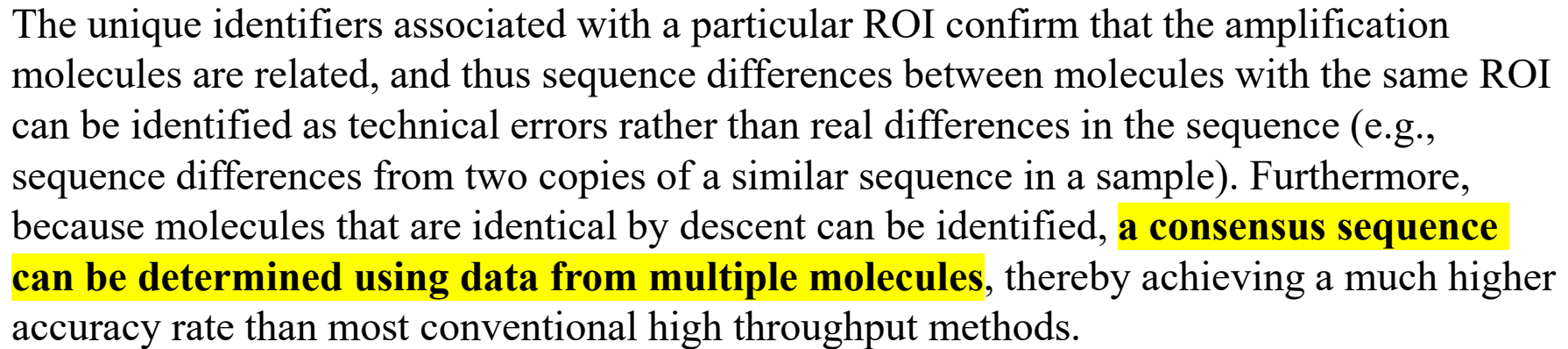
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Claims 1 & 2

1. A method for correcting for errors or bias... comprising the steps of:

correcting for error or bias introduced during said workflow by **collapsing target:differentiator tag combinations observed more than once into a single count**.

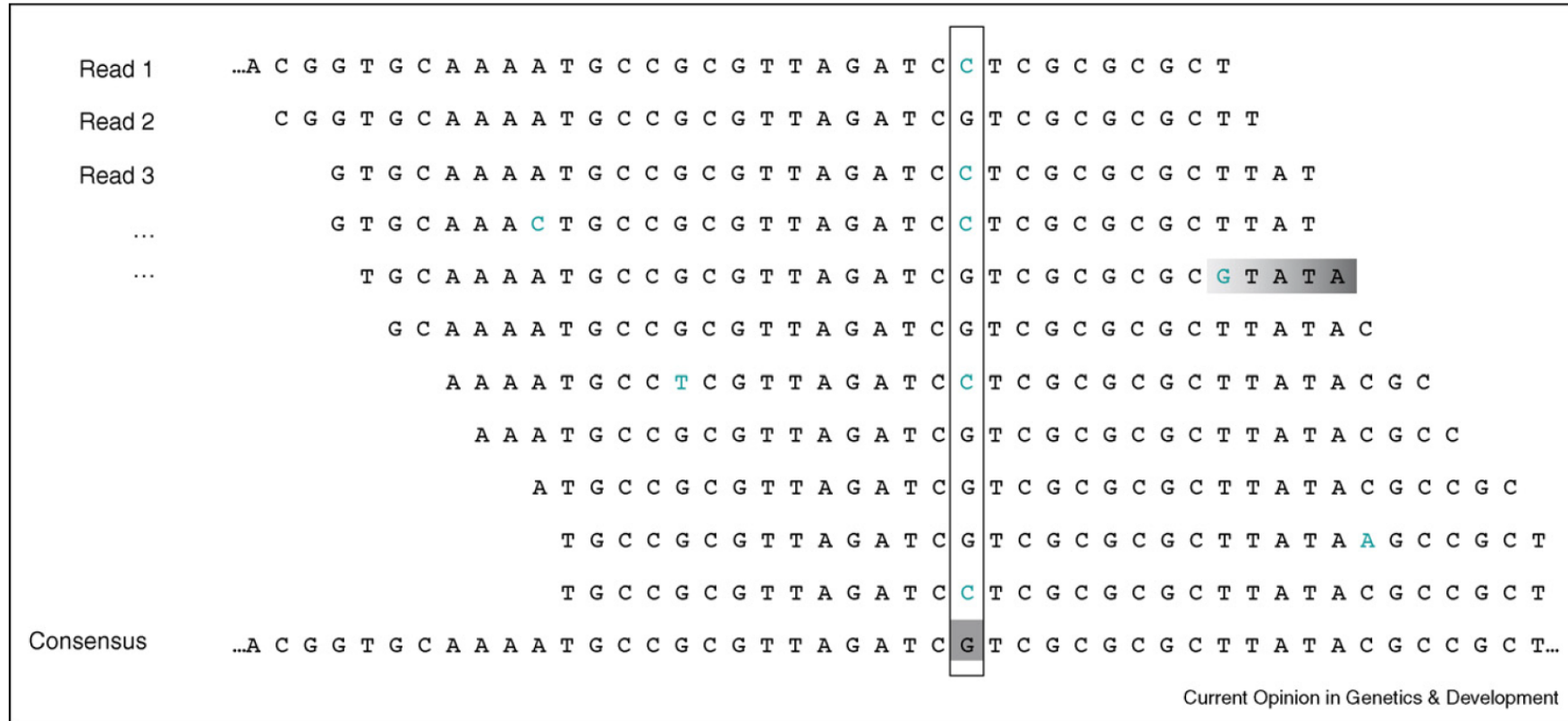
2. The method of claim 1, wherein **said correcting step** further comprises **collapsing all sequence reads comprising a same differentiator tag into a single read** before determining **a consensus base call** for the target nucleic acid molecule sequences.

Chee

EX1010, 9:25-39; Pet., 25, 32
See also EX1010, 14:42-51

Bentley: Consensus Basecall

Figure 2



EX1017, 550

For example, **multiple reads** from the same sample can be aligned and used to derive **a consensus base-call** at each position.

EX1017, 549; Pet., 19-20

ML's Own Expert Admits Chee Derives a Consensus Sequence as Described by Bentley



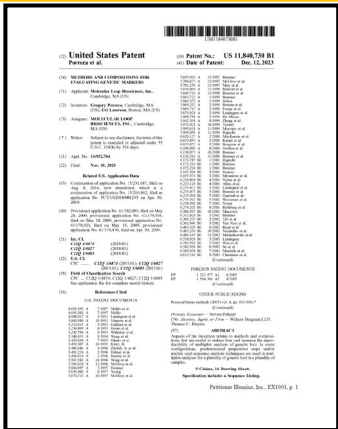
Dr. Puglisi
ML's Expert

Q In column 14, line -- at line 48, **Chee** uses the phrase "consensus sequence derived from multiple clonal copies of the construct." How would you understand creating **a consensus sequence derived from multiple clonal copies** of the construct?

A At each position throughout the sequence, **he's doing what Bentley did**. That's the way I read it.

EX1066, 113:3-9; Reply, 14

Chee Collapses Reads and Generates a Consensus



2. The method of claim 1, wherein said correcting step further comprises collapsing all sequence reads comprising a same differentiator tag into a single read **before** determining a consensus base call for the target nucleic acid molecule sequences.



Dr. Gabriel
Illumina's Expert

26. Chee's process works in two steps. **First**, Chee identifies constructs that have the same unique identifier and groups them as being identical by descent. EX1010(Chee), 9:29-39. **This grouping "collapses" the identical constructs because the bin represents a single count** of the ROI in the original sample. **Chee then uses the multiple reads of the grouped constructs to generate a consensus sequence.** EX1010(Chee), 9:29-39. Although claim 2 has two steps, the two steps can be part of the same process.

EX1065, ¶126; Reply, 15

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Gahan Teaches that DNA Extracted from Blood Has Been Used for Diagnostic Purposes

Gahan

Circulating Nucleic Acids in Plasma and Serum

Recent Developments

Peter B. Gahan* and R. Swaminathan†

*Department of Chemical Pathology, King's College, London, London, United Kingdom
†NRI Foundation Trust, London, United Kingdom

DNA and RNA fractions have been isolated from the whole blood, serum, plasma, the surface of blood cells, and tissue of both healthy individuals and patients. The ability to isolate, quantify, and analyze these nucleic acids has led to the identification of specific nucleic acid fragments related to particular diseases such as diabetes, cancer, neurological disorders, and various other genetic diseases. Currently, a number of methods for isolating the nucleic acids are employed and although a great deal has been made to compare the effectiveness of these methods, there is still a way to go before there are generic protocols for nucleic acid extraction. The other chemical methods of extraction still require some of the available kits. Some progress is being made to determine the origin of the circulating nucleic acids, although there are still many questions to be answered, including whether the source is through the spontaneous release of newly synthesized nucleic acid or whether it just derived from necrotic and apoptotic cells. In addition, it can be demonstrated that the nucleic acids can enter cells and exhibit a biological activity in the recipient cells. Hence, the question remains: Are the circulating nucleic acids truly carrying messages and cells from the blood and inducing changes in these tissues and cells? Further work is needed to elucidate these issues, and the various protocols must be standardized if the new methodology is to be widely and accurately applied in the diagnosis of disease and the monitoring of therapy. This chapter summarizes the work reported in this volume.

Key words: circulating nucleic acids; CNAPS in oncology; CNAPS in fetal medicine; biology of CNAPS; CNAPS in genetic medicine

Introduction

The conference celebrated the 60th anniversary of the first report of circulating DNA by Minetti and Meites in 1947. After a long time, nucleic acids in plasma and serum were found to be present in the 1970s and 1976 for the spontaneous release of newly synthesized DNA by living cells (reviewed in Swaminathan¹). In parallel, increased DNA in the blood of patients with systemic lupus erythematosus was demonstrated.² Evidence in the 1970s by studies on increased DNA levels in blood from patients with other disorders such as paraneoplastic and inflammatory bowel disease.³ Higher levels of DNA were also found in blood from cancer patients,⁴ especially in those with metastases compared to patients with

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DOI: 10.1191/14732165.2008.019153

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doi: 10.1191/14732165.2008.019153

† Peritomer Illumina, Inc., EX1015, p. 1

Many studies have shown **the successful application** of [Circulating Nucleic Acids in Plasma] in the **diagnosis and monitoring of cancer and in prenatal diagnosis, as well as in other diseases**. However the methods used for the extraction and quantification of nucleic acids vary widely.

EX1010, 11:51-54; Pet., 21; Reply, 17

37. Dr. Puglisi also overlooks the disclosure in Gahan which recognizes that cell-free DNA extracted from blood had been used for diagnostic purposes. For instance, Gahan states that “[m]any studies have shown the successful application of CNAPS [circulating nucleic acids in plasma and serum] in the diagnosis and monitoring of cancer and in prenatal diagnosis, as well as in other diseases.” EX1015(Gahan), 3. Therefore, **a POSA would have recognized that the nucleic acids isolated from blood as Gahan could be used with Chee, especially since Chee discloses that its methods can be used for diagnostic purposes**. E.g., EX1010(Chee), 11:60-63.

EX1065, ¶137; Reply, 16-17



Dr. Gabriel

Illumina's Expert

CheePetitioner Illumina, Inc., EX1010, p.

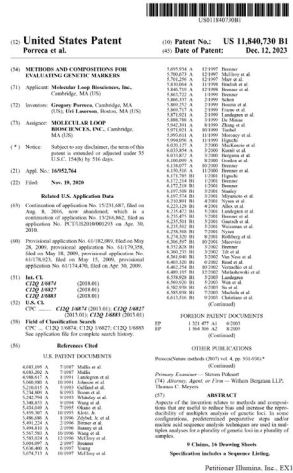
EX1010, 11:51-54; Pet., 21; Reply, 17

EX1010, 11:51-54; Pet., 21; Reply, 17

Disputed Issues

- Grounds 4-7: McCloskey + Bignell
 - Whether McCloskey teaches the “collapsing” step of claim 1
 - Whether there is motivation to combine McCloskey and Bignell
- Grounds 1-3: Chee
 - Whether Chee teaches the “collapsing” step of claim 1
 - Whether Chee teaches the “consensus base call” of claim 2
 - Whether there is motivation to combine Chee with Gahan for claims 5, 6, and 8
 - **Whether Molecular Loop has shown Chee is not prior art**
 - **Whether Chee’s provisional application discloses a “biological sample” such that the Chee reference is entitled to its provisional application date**
 - Whether Molecular Loop has shown prior conception of claims 1-3 and 9
 - Whether Molecular Loop has shown reasonable diligence for claims 1-3 and 9

'730 Claim 1



1. A method for correcting for errors or bias introduced during nucleic acid analysis workflow, the method comprising the steps of:

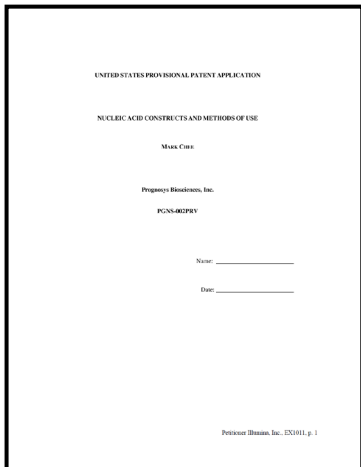
- obtaining **a biological sample** comprising a plurality of target nucleic acid molecules from more than one locus of origin;
- introducing a set of differentiator tags, wherein members of said set of differentiator tags are associated with members of said plurality, such that one or more of said loci of origin are associated with more than one differentiator tag;
- amplifying each of the plurality of tagged target nucleic acid molecules to generate amplicons;
- sequencing the amplicons obtained in said amplifying step to obtain sequence reads of each of the amplicons, wherein each of the sequence reads comprises a target nucleic acid molecule sequence and a differentiator tag sequence; and
- correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

Chee's Provisional Expressly Discloses "Biological Samples"

[00060] The invention has an advantage over conventional techniques for error detection, as it addresses two primary sources of error encountered in conventional synthetic biology and analysis of nucleic acids in **biological samples** - that is, errors in nucleic acid isolation/production and errors introduced in amplification of the original nucleic acid starting materials.

EX1011, [00060]; Pet., 27

Chee Provisional



[00066] Although the present invention is primarily described in the context of synthetic biology and quantitative analysis, the constructs and methods **can be used more generally** to identify and obtain any desired sequence from a collection of nucleic acid molecules. For example, the methods of the invention can be used to create a large collection of smaller nucleic acids with extremely high fidelity for any discovery research, **diagnostic use, clinical use** or other development techniques.

EX1011, [00066]; Pet., 27

ML's Own Expert Understands "Clinical" and "Diagnostic" to Refer to Human Use



Dr. Puglisi
ML's Expert

Q Does "clinical" refer to patient care?

A I mean, **"clinical" involves human patients and diseases**. Whether it leads to improved patient care, obviously, that's the goal....

EX1066, 32:15-18; Reply, 19

Q Do you understand **"diagnosis"** to refer to something that's used on a human patient?

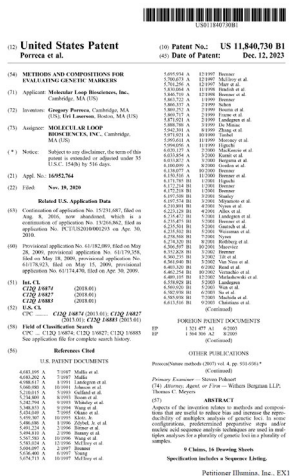
A Again, I would imagine -- I mean, obviously, diagnosis can occur on plants if you're in agriculture, in veterinary settings if you're a veterinarian, but I think **the meaning here is targeted towards human patients, yes**. But it has a broader sense than that.

EX1066, 34:14-20; Reply, 19

Disputed Issues

- Grounds 4-7: McCloskey + Bignell
 - Whether McCloskey teaches the “collapsing” step of claim 1
 - Whether there is motivation to combine McCloskey and Bignell
- Grounds 1-3: Chee
 - Whether Chee teaches the “collapsing” step of claim 1
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 - **Whether Molecular Loop has shown Chee is not prior art**
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'730 Claim 1



1. A method for **correcting for errors or bias** introduced during nucleic acid analysis workflow, the method comprising the steps of:

- obtaining a biological sample comprising a plurality of target nucleic acid molecules from more than one locus of origin;
- introducing a set of differentiator tags, wherein members of said set of differentiator tags are associated with members of said plurality, such that one or more of said loci of origin are associated with more than one differentiator tag;
- amplifying each of the plurality of tagged target nucleic acid molecules to generate amplicons;
- sequencing the amplicons obtained in said amplifying step to obtain sequence reads of each of the amplicons, wherein each of the sequence reads comprises a target nucleic acid molecule sequence and a differentiator tag sequence; and
- correcting for error or bias** introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

Illumina's Expert: "Errors" and "Bias" Are Different



Dr. Gabriel
Illumina's Expert

39. Claim 1 recites a method of “correcting for errors or bias”. As I explained in my prior declaration, a POSA would have understood **errors and bias to refer to different problems** that can arise in the nucleic acid workflow. EX1005, ¶¶ 75-77. The claims separately recite error and bias, which I interpret as meaning that **an error is something distinct from bias**.

EX1065, ¶39; Reply, 22

40. **Errors** can occur **when nucleotides are incorrectly incorporated when making a copy of DNA** by synthesizing a complementary strand.... Sequencing error also can occur when the nucleotide was correctly incorporated in the complementary strand, but **subsequently mis-read by the sequencing machine**. A POSA would consider these to be errors—not bias. In contrast, **bias** generally refers to **uneven amplification of target molecules** which results in some molecules being over- or under-represented following amplification as compared to the original sample. In the context of the claims, **bias is distinct from error**.

EX1065, ¶40; Reply, 22

ML's Expert: "Errors" and "Bias" Are Different



Dr. Puglisi
ML's Expert

Q In the context of claim 1 of the '730 patent, **is there a difference between bias and error?**

A Well, they say error or bias, so --

Q How is error different than bias in the context of claim 1?

A An **error** could be an error that happens in your sample workup. Okay? It could be that, you know, whatever nucleic acid polymerase or, you know, other manipulation enzymes that you use to prepare the sample led to incorrect nucleotides or **changes in the sequence** happening during that process.

That would be an error that was introduced in the process, whereas **bias** is, you're **not changing the sequence**, you're **changing the relative populations of the sequences**.

So they are different things, but I think what **the claim is stating is they are different than one another**.

EX1066, 95:22-96:17; Reply, 22

ML's March 2009 Application Describes Only Correcting for Bias

1. A method for **correcting for errors or bias** introduced during nucleic acid analysis workflow, the method comprising the steps of:

* * *

correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

March 6, 2009 Draft Application Priority Disclosure:

METHODS FOR REDUCING BIAS IN PREPARATIONS OF NUCLEIC ACIDS

Summary of the Invention/Detailed Description [We can break it up later.]

The invention is the composition and use of a large library of unique molecular barcodes in preparative steps for reducing the effects of the bias described above. During the library preparation process, each molecule is tagged with a unique in such a configuration such that the barcode is sequenced along with the information of interest for which the library is being prepared. The incorporation of the barcode at a given step allows the detection and correction of biases in any of the following steps of the protocol.

EX2006, 1-2; Reply, 21

ML's March 2009 Application Does Not Describe Correcting for Errors

1. A method for **correcting for errors or bias** introduced during nucleic acid analysis workflow, the method comprising the steps of:

* * *

correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

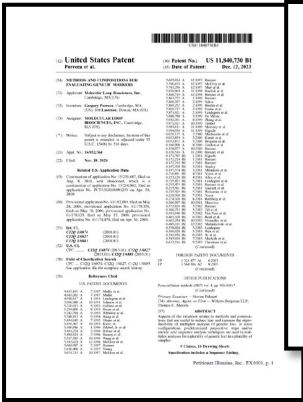
March 6, 2009 Draft Application Priority Disclosure:

METHODS FOR REDUCING BIAS IN PREPARATIONS OF NUCLEIC ACIDS

Finally, there is an approach to utilize this random barcode methodology retrospectively on samples that do not explicitly incorporate unique barcodes. Most oligonucleotide synthesis methods are error-prone. The fraction of oligos with errors depends on the length of the oligonucleotide and the synthesis method employed. For example, using traditional column synthesis of 25-mers, we would expect that 1 in 40 PCR primers would contain a **synthesis error** (assuming one error every 1,000 bases). These error-containing primers are also incorporated in the PCR reaction. Since the errors are distributed in a random fashion, they are unique and rare; therefore, the error-containing PCR primers can be considered as unique random barcodes as well. Since these primers typically get sequenced as well, the errors can be detected and **used as random barcodes to look for biases** in the same way described above.

EX2006, 1-2; Reply, 21

ML's March 2009 Application Describes Only Correcting for Bias

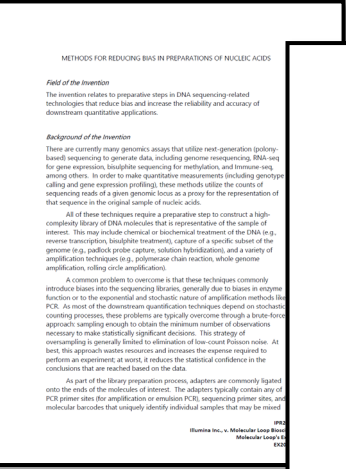


1. A method for **correcting for errors or bias** introduced during nucleic acid analysis workflow, the method comprising the steps of:

* * *

correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

Patent Owner's Conception Claim Chart:



Claims	March 6 Application
Claim 1: A method for correcting error or bias introduced during nucleic acid analysis workflow, the method comprising the steps of:	“The invention relates to preparative steps in DNA sequencing-related technologies that reduce bias and increase the reliability and accuracy of downstream quantitative applications.” EX2006 at 1. EX2023, ¶¶67, 84; <i>see also</i> EX2023, ¶¶86, 99.

PO Response, 24

ML's March 2009 Application Describes Only Correcting for Bias

Patent Owner's Conception Claim Chart:

correcting for error or bias

introduced during said workflow by collapsing target: differentiator tag combinations observed more than once into a single count.

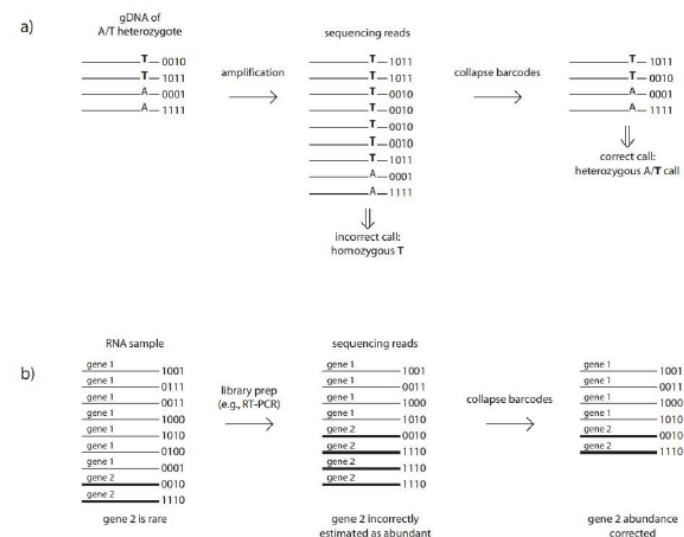
"[B]y observing how the distribution of random barcodes changes after the sequencing is performed, we have collected the information that is necessary to correct the biases. By undoing the distribution changes that are measured for the random barcodes, the corresponding changes in the counts for the sequences of interest will provide a higher fidelity representation of the composition of the original complex pool." EX2006, 3.

"However, if we also have information on random barcodes, this situation could be averted, because the over-represented allele would be seen to also have an over-represented random barcode (i.e., the sequences with the overrepresented barcode all originated from the same single molecule). Therefore, the sequences and corresponding distribution of random barcodes can be used as an additional input to the genotype-calling algorithm to significantly improve the accuracy and confidence of the genotype calls." EX2006, 5.

"The incorporation of the barcode at a given step allows the detection and correction of biases in any of the following steps of the protocol." EX2006, 2.

"The observations of the random barcode sequences are used to detect and correct biases in the final read-out of the library." EX2006, 2.

"[I]f each RNA/cDNA molecule is coupled to a unique random barcode sequence, then any repeated barcodes in the sequencing reactions can simply be collapsed into each other. By collapsing similar clones in such a way that the sequencing data only includes a single representative for each barcode (or barcode-molecule combination), the biases that were introduced in preparation are essentially eliminated." EX2006, 5.



EX2008.

EX2023, ¶¶72, 84; *see also* EX2023, ¶¶91, 99.

PO Response, 28-29

“Systematic Errors (Bias)” First Appears in May 2009

1. A method for **correcting for errors or bias** introduced during nucleic acid analysis workflow, the method comprising the steps of:

* * *

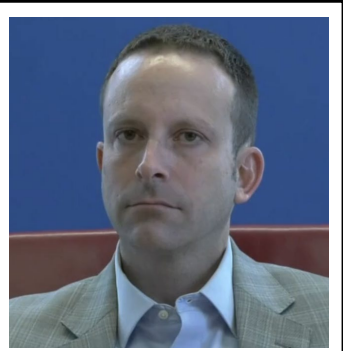
correcting for error or bias introduced during said workflow by collapsing target:differentiator tag combinations observed more than once into a single count.

May 14, 2009 Revised Draft Application:

Systematic errors are common problems associated with genome amplification and sequencing library construction techniques. For example, genomic sequencing library may contain an **over- or under-representation** of particular sequences from a source genome as a result of **systematic errors (bias)** in the library construction process. **Such bias** can be a particularly problematic when it results in target sequences from a genome being absent or undetectable in the sequencing libraries. For example, an under-representation of particular expense required to perform such experiments. Moreover, oversampling can result in a reduced statistical confidence in certain conclusions (e.g., diagnostic calls) based on the data. **Accordingly, new approaches are needed for overcoming bias** in sequencing library preparatory methods.

EX1048, 3-4

Dr. Porreca Did Not Know the Relationship Between Bias and Error



Dr. Porreca
Named Inventor

Q. So **you can't tell me the difference, if any, between bias and error** in this application?

A. **I'm just not prepared** to discuss specific terms as they were used in the patent.

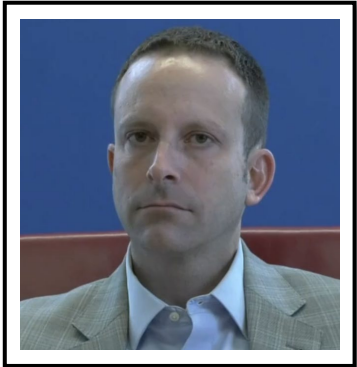
EX1068, 144:12-17; Reply, 22

Q. Okay. **Is bias the same as systematic error?**

A. **I don't know.**

EX1068, 139:16-18; Reply, 22

Dr. Porreca Did Not Know the Relationship Between Bias and Error



Dr. Porreca
Named Inventor

Q. I'm just asking you: **Does Claim 1 recite an invention that corrects for bias?**

A. **It recites what it recites.**

Q. Is that a yes or a no?

A. That is a **I cannot give you an answer about specific words, like error or bias**, without reviewing the entire patent as a whole, and I have not done that in preparation for today, and I'm not prepared to do that today.

Q. **Does Claim 1 recite an invention that corrects for errors?**

A. That's going to be **the same answer.**

Q. Is there **any difference between errors and bias** in the context of Claim 1?

A. Again, it's going to be the same. It's the same. We're in the **same situation**

EX1068, 150:22-151:16; Reply, 22

Disputed Issues

- Grounds 4-7: McCloskey + Bignell
 - Whether McCloskey teaches the “collapsing” step of claim 1
 - Whether there is motivation to combine McCloskey and Bignell
- Grounds 1-3: Chee
 - Whether Chee teaches the “collapsing” step of claim 1
 - Whether Chee teaches the “consensus base call” of claim 2
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 - Whether Molecular Loop has shown prior conception of claims 1-3 and 9
 - **Whether Molecular Loop has shown reasonable diligence for claims 1-3 and 9**

Pre-Filing Timeline: March 6 to May 18, 2009

Reply, 25-27

March

April

May

Pre-critical : 7+ Weeks of Work on Application

Critical Period

Mar. 6
Porreca sends draft
application to
attorneys

Apr. 23
Attorneys state
they have all info
needed

Apr. 30
Chee's
provisional filed

May 18
Porreca's
provisional filed

From: [Eric Boutin](#)
To: ["Patrick Waller"](#)
Cc: [greg.porreca@gmail.com](#); [ulaserson@gsgenetics.com](#); ["Bob Wassman"](#); ["Paris Wallace"](#); [jin.billy.li@gmail.com](#)
Subject: Good Start Genetics - IP Meeting Wed 11th at 9am
Date: Friday, March 6, 2009 3:11:09 PM
Attachments: [Bias-detecting_Barcodes_v2.pdf](#)
[gsa_patapp_reduce-amp-bias2.pdf](#)
[ProvIP_MIP_length_20090307.pdf](#)
[bias_detecting_barcodes_fig1.pdf](#)
[bias_detecting_barcodes_fig2.pdf](#)

Hello Patrick-

Please find drafts of our three provisional patent applications attached. Below is also a list of papers and patents associated with the drafts.

EX2021, 1

From: [Waller, Patrick](#)
To: [Greg Porreca](#); [Waller, Patrick](#); [Young, Dan](#)
Cc: [Eric Boutin](#)
Subject: RE: FW: Target Table
Date: Thursday, April 23, 2009 9:04:26 AM

Hi Greg,
I think that you have sent us all of the important information that we need. Dan or I will send you updated drafts today for your comment and feedback. They will include a few questions on issues that we want to clarify, but they should not need significant technical input at this stage.
Patrick

EX1054, 1

Molecular Loop Failed to Show Reasonable Diligence During 20-day Critical Period

Reply, 25-27

March

April

May

Pre-critical : 7+ Weeks of Work on Application

Critical Period

Mar. 6 to Apr. 28

Apr. 29 – May 18

Date	Inventor Activity	Attorney Activity
4/29/09	Phone call between Young and Porreca. EX2012, 2-3.	
4/30/09	Porreca sends Waller inventorship information.	No evidence of activity.
5/1/09		No evidence of activity.
5/2/09		No evidence of activity.
5/3/09		No evidence of activity.
5/4/09		No evidence of activity.
5/5/09	Porreca sends two paragraphs to Young for inclusion in provisional.	Young asks Porreca a question. Waller call with Porreca. EX2014, 4.
5/6/09		No evidence of activity.
5/7/09		No evidence of activity.
5/8/09		No evidence of activity.
5/9/09	Phone call between Waller and Porreca. EX2014, 2.	
5/10/09		No evidence of activity.
5/11/09	Porreca stated "Uri's [application] probably doesn't need further revision."	No evidence of activity.
5/12/09		No evidence of activity.
5/13/09		No evidence of activity.
5/14/09	No evidence of activity.	Young sends draft of '358 Provisional to Porreca.
5/15/09	Porreca sends edits to Young.	No evidence of activity.
5/16/09		No evidence of activity.
5/17/09		No evidence of activity.
5/18/09		'358 Provisional filed.

In re Mulder



The Board cites *In re Mulder* for the proposition that “[e]ven a short period of unexplained inactivity may be sufficient to defeat a claim of diligence.” ... Although the critical period spanned just two days, we declined to excuse the patent owner’s complete lack of evidence.

Perfect Surgical Techniques, Inc. v. Olympus America, Inc., 841 F.3d 1004, 1009 (Fed. Cir. 2016)
(discussing *In re Mulder*, 716 F.2d 1542 (Fed. Cir. 1983))

Dr. Waller's Declaration Should be Given No Weight



Dr. Waller
Prosecuting
Attorney

Q. Do you recall reviewing the draft application that Dr. Young sent to Dr. Porreca on May 14, 2009?

A. I don't recall doing that, no. I assume that I did.

EX1067, 55:2-6; Reply, 24

Q. Do you recall drafting any portion of what became the '358 provisional application?

A. No. And by that, I mean I don't remember. I just don't know.

EX1067, 57:17-20; Reply, 24

Reserve

'730 Patent: "Collapsing Differentiator Tag Sequences"

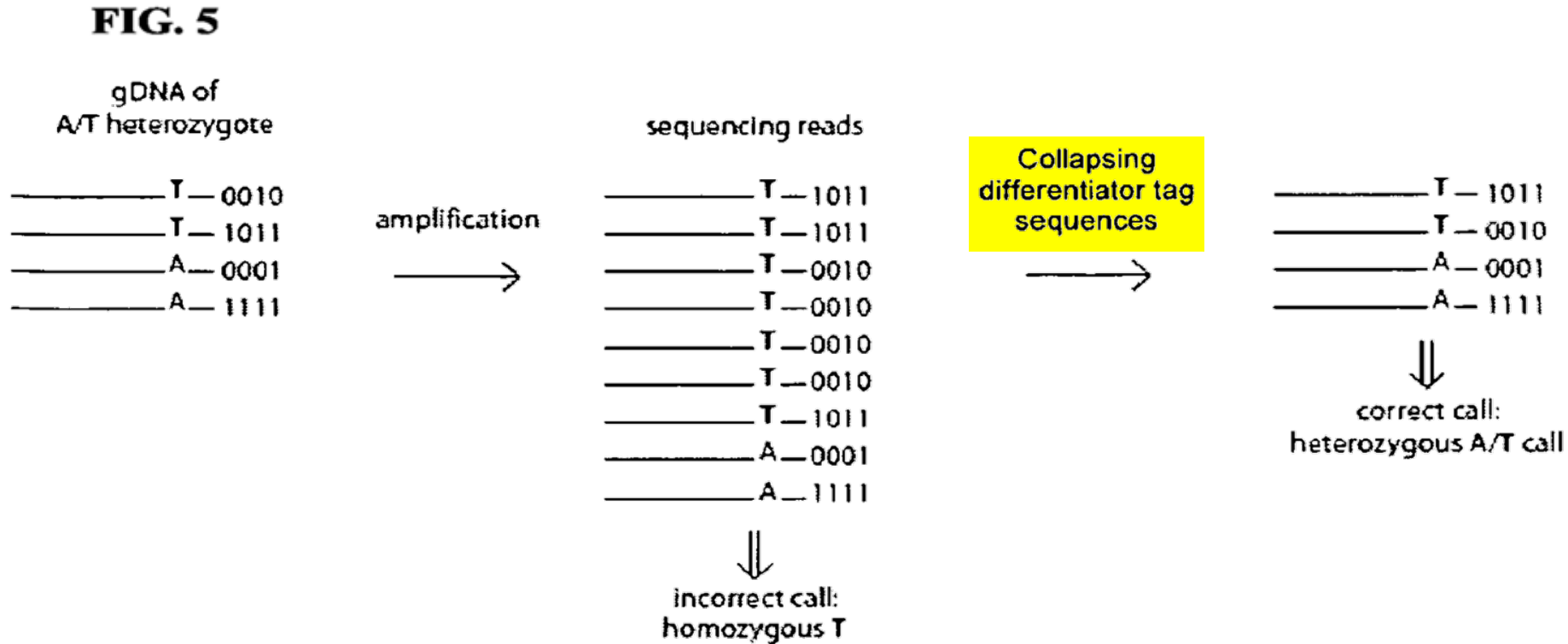
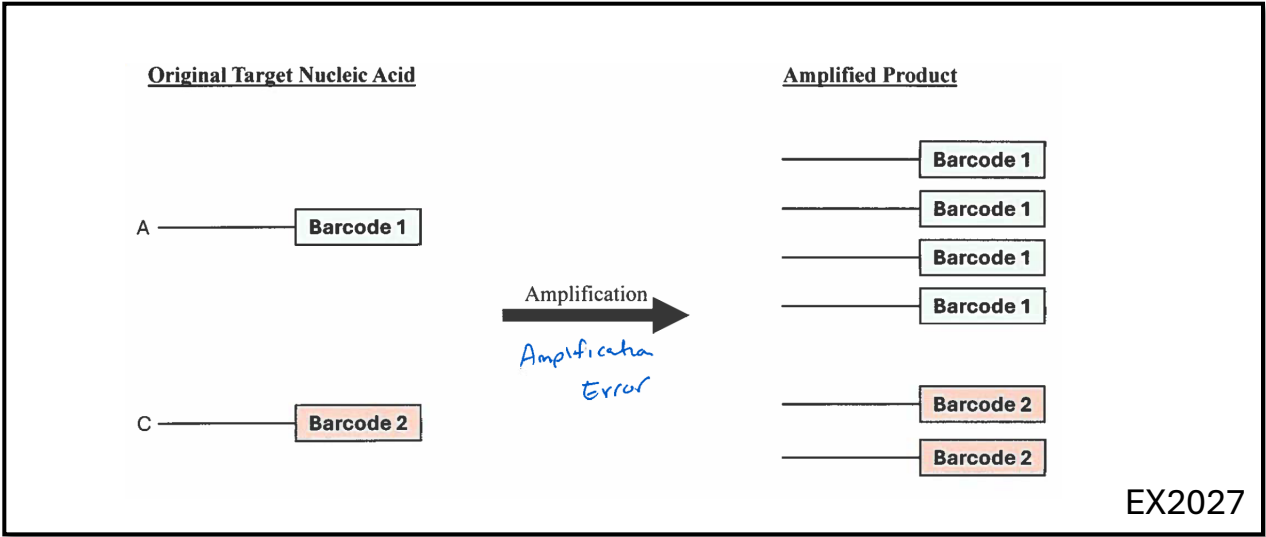
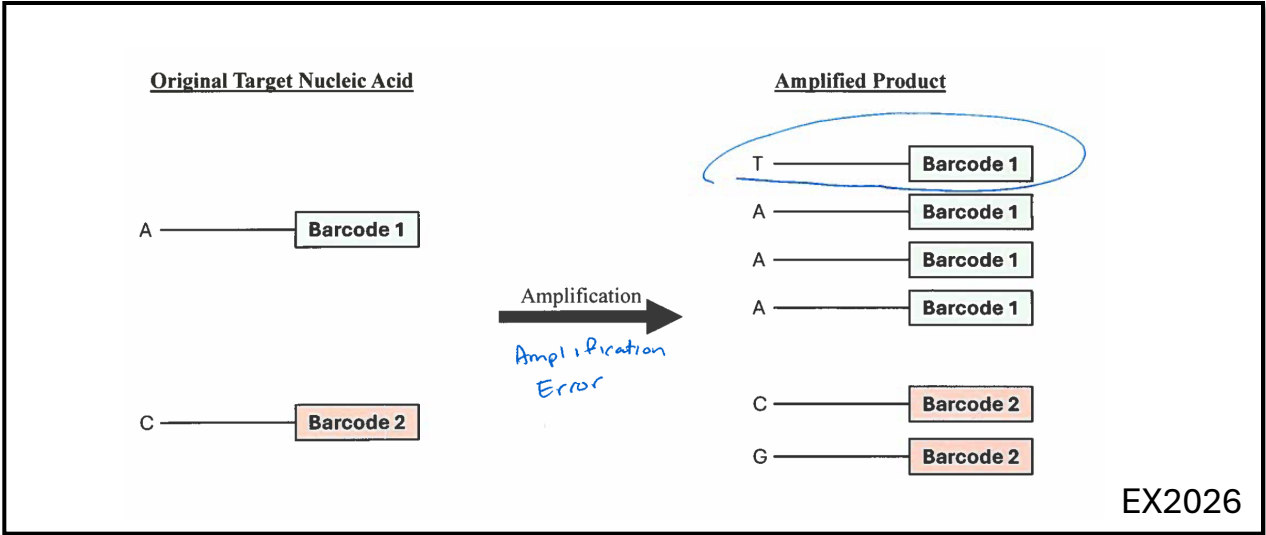
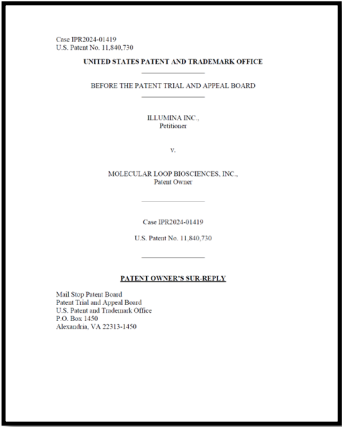


FIG. 5 depicts a method for making diploid genotype calls in which **repeat target:differentiator tag combination are collapsed**.

EX1001, 73:3-5; Reply, 5-6

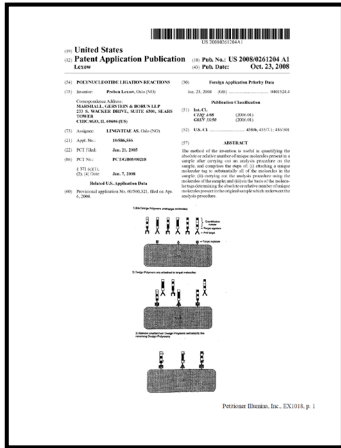
ML's Hypotheticals Are Incomplete and Inaccurate

Patent Owner Sur-Reply



Lexow Used Unique Tags to Solve the Same Problem with Amplification Bias

Lexow



For example, in **an amplification process one target molecule in a sample**

may be copied more times than another target molecule, thereby making it

difficult to measure the absolute and relative amounts of the different target molecules that were present in the original sample. Furthermore, the analysis procedure itself often results in the mixing of molecules such that it is not possible to maintain information on each individual molecule.

EX1018, [0002]; Reply, 11

The present invention is based on the realisation that **the absolute and/or relative amounts of a unique target molecule** can be determined and that individual molecules within a population can be tracked throughout an analysis procedure, by **using a molecular tag that is unique to each specific molecule**.

EX1018, [0005]; Reply, 11

ML's Own Expert Admits Chee Corrects Amplification Bias



Dr. Puglisi
ML's Expert

Q **Chee** recognizes that **skewing during amplification** can be a problem?

A **Yes**. We've already discussed that **this was a well-known problem** in the field. So Chee was definitely aware of this issue.

EX1066, 120:1-5; Reply, 1, 13

Q So ignoring whether the source is natural or synthetic DNA, you agree that **Chee discloses correcting for amplification bias**, correct? ...

[**A**]: He -- what Chee is proposing is a method -- obviously, he needs to show that that method works, but **by just reading the unique identifiers, he is proposing that that would allow him to correct for amplification bias**.

EX1066, 122:12-20; Reply, 13

Molecular Loop Argues the Claims Require Determining a Genotype

Case IPR2024-01419
U.S. Patent No. 11,840,730
UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA INC.,
Petitioner

v.

MOLECULAR LOOP BIOSCIENCES, INC.,
Patent Owner

Case IPR2024-01419
U.S. Patent No. 11,840,730

PATENT OWNER'S RESPONSE

Mail Stop Patent Board
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Patent Owner Response:

Figure 5 shows that, when certain starting molecules are over- or under-amplified and redundant sequence reads are not removed, the skewed population of amplicons will result in **generating a consensus base sequence using all amplicons and thus causing an incorrect genotype call**... Instead, as shown in teal below, **when identical sequence reads are collapsed prior to generating a consensus base call**, errors arising during amplification bias will be eliminated from the calculations, **resulting in a correct genotype call**...

PO Resp, 44

[A] POSA would have understood that the inclusion of the target nucleic acid molecule when **collapsing** target:differentiator tag combinations **allows a POSA to make diploid genotype calls** and lays the groundwork for **more accurate consensus basecalls**.

PO Resp, 53

CheePetitioner Illumina, Inc., EX1010, p.1

EX1010, 9:42-52; Pet., 26; Reply, 16-17

EX1065, ¶28; Reply, 16-17