

PATENT TRIAL AND APPEAL BOARD
UNITED STATES PATENT AND TRADEMARK OFFICE

Illumina, Inc.
v.
Molecular Loop Biosciences, Inc.

September 23, 2025

IPR2024-00964 U.S. Patent No. 11,041,852
IPR2024-00965 U.S. Patent No. 11,768,200

The logo for Molecular Loop Biosciences, Inc. features the word "molecular" in a dark blue, lowercase, sans-serif font, followed by the word "loop" in a lighter blue, lowercase, sans-serif font. The "o" in "loop" is stylized with a circular arrow graphic around it, suggesting a loop or cycle.

IPR2024-00964 & 2024-00965
Illumina Inc., v. Molecular Loop Biosciences,
Inc. Molecular Loop's Exhibit 2016

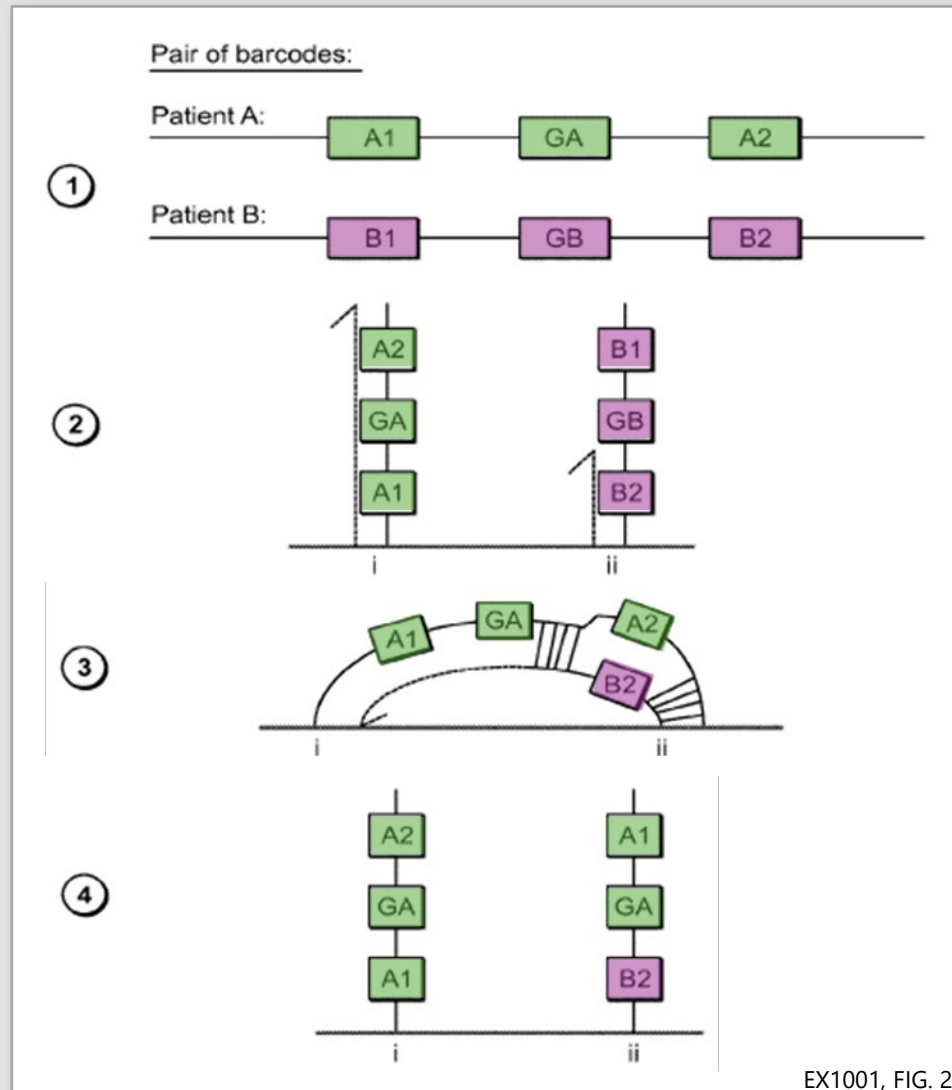
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Outline

- A** Overview of the Molecular Loop Patents
- B Overview of the Prior Art
- C Disputed Issues

The Molecular Loop Patents Introduced A New Method For Dual Indexing To Detect Invalid Sequences In Multiplex Sequencing



"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 FIG. 1, ***the chimeric molecule formed in this example includes one barcode sequence from patient A (A1) and one barcode sequence from patient B (B2)*** ... 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.***"

964 IPR EX1001 ('852 Patent), 5:52-6:4; 965 IPR EX1001 ('200 Patent), 5:52-6:4.

Outline

- A Overview of the Molecular Loop Patents
- B Overview of the Prior Art
- C Disputed Issues

Prior Art: Parameswaran Discloses Using Two Identifiers But Keeping All Samples That Have A Match To At Least One Identifier

Parameswaran recognized that “[m]ultiplexed high-throughput pyrosequencing is currently limited in complexity (**number of samples sequenced in parallel**), and in capacity (**number of sequences obtained per sample**).”

EX1004, Abstract.

Parameswaran used two unique barcode motifs for each sample to assign sequences to a given sample based on the presence of **either barcode motif**.

EX1004,11-12 (“Sequences that have been recognized to contain either or both motifs are FASTA-formatted, and placed in their experiment’s ‘raw’ bin. Additionally, these sequences are stripped of their barcode motifs, and placed in their experiment’s ‘trimmed’ bin.”); *see also* EX2015, 115:15-116:11, 117:22-118:2, 118:12-119:2.

Parameswaran concludes that “over 99.8% of obtained sequences could be assigned to 25 independent, uniquely barcoded libraries based on the presence of **either a perfect forward or a perfect reverse barcode**.”

EX1004, Abstract.

Parameswaran specifically identifies a “remarkably low misassignment rate” as a reason to explore the “potential utility of **combinations of forward and reverse barcodes** to generate up to 48^2 (= 2304) combination barcodes for this set of sequences.”

EX1004, 8.

Prior Art: Gloor Discloses Using Non-Unique Dual Indexing, Which Repeats The Same Individual Identifiers Across Multiple Different Samples

Gloor used a total of 34 unique sequence tags (18 left sequence tags and 16 right sequence tags) in a combinatorial manner to create unique pairs of sequence tags that are used to sequence 272 samples together in a single sequencing run.

EX1005, 2, 6.

Gloor explains that “combinatorial sequence tags allows the investigator to examine ***hundreds of samples*** with ***far fewer primers*** than is required for single-end bar-code sequencing.”

EX1005, 22.

Gloor touted its sequencing accuracy, explaining “the Illumina sequencing platform has a ***substantially lower error rate than does the 454 pyrosequencing platform.***”

EX1005, 12.

Prior Art: Bentley Discloses Single Sample Whole-Genome Sequencing Using Illumina Sequencing Platforms

Bentley discloses a method that utilizes Illumina sequencing platforms to “determine the genome sequence of a male Yoruba from Ibadan, Nigeria.”

EX1006, Abstract.

Bentley’s approach “demonstrate[s] utility by re-sequencing an individual human genome to high accuracy” but does not discuss or suggest multiplex sequencing, pooling, or sample tagging.

EX1006, 1.

Prior Art: Binladen Discloses Using The Same Sequence Identifier With A Given Sample To Correctly Assign Sequences

Binladen discloses a pyrosequencing method that allows each DNA sequence to be “subsequently traced back to its individual source ***through 5’ tag-analysis.***”

EX1029, Abstract.

Because pyrosequencing reads were limited to “approximately 120 bases” and the full sequence in Binladen was 133–141 bp, it acknowledges that full sequences with tag sequences on both ends may not be observed.

EX1029, 3.

Binladen therefore uses the same barcode on either end of a template nucleic acid to ensure that one barcode is analyzed.

EX1029, 2, 3.

Outline

- A Overview of the Molecular Loop Patents
- B Overview of the Prior Art
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Petitioner's Grounds

IPR2024-00964 ("964 IPR")

Ground	Challenged Claims	References
1	1-8	Parameswaran and Gloor
2	1-8	Parameswaran and Bentley

IPR2024-00965 ("965 IPR")

Ground	Challenged Claims	References
1	1-6	Gloor
2	1-6	Parameswaran
3	1-6	Parameswaran and Gloor
4	1-6	Parameswaran and Binladen
5	6	Parameswaran, Binladen, and Bentley

Disputed Issues – 964 IPR

- 1 The intrinsic evidence confirms PO's construction of the "cross-over error" term.
- 2 Lack of motivation to combine Parameswaran and Gloor. (Ground 1)
- 3 Lack of reasonable expectation of success for the combination of Parameswaran and Gloor. (Ground 1)
- 4 Petitioner does not show that the combination of Parameswaran and Gloor discloses or renders obvious the "discarding" step. (Ground 1)
- 5 Petitioner does not establish Gloor as prior art. (Ground 1)
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Patent Owner's Construction Of "Cross-Over Error" Is Supported By The Intrinsic Record And Is Relevant To Petitioner's Obviousness Grounds

Patent Owner's Construction

"error from sequences of chimeric molecules created during cluster amplification and made from template nucleic acids derived from different samples"

964 IPR POR, 13-14; 964 IPR EX2001, ¶¶24-25

Patent Owner's Construction Of "Cross-Over Error" Is Supported By The Claim Language

- The '852 Patent describes methods for identifying and reducing error, such as "false positives" and "false negatives," for more accurate analysis. *See* EX1001, 7:37-38.
- Patent Owner's construction accurately reflects that concept when inserted into the claims.

'852 Patent Claim 1

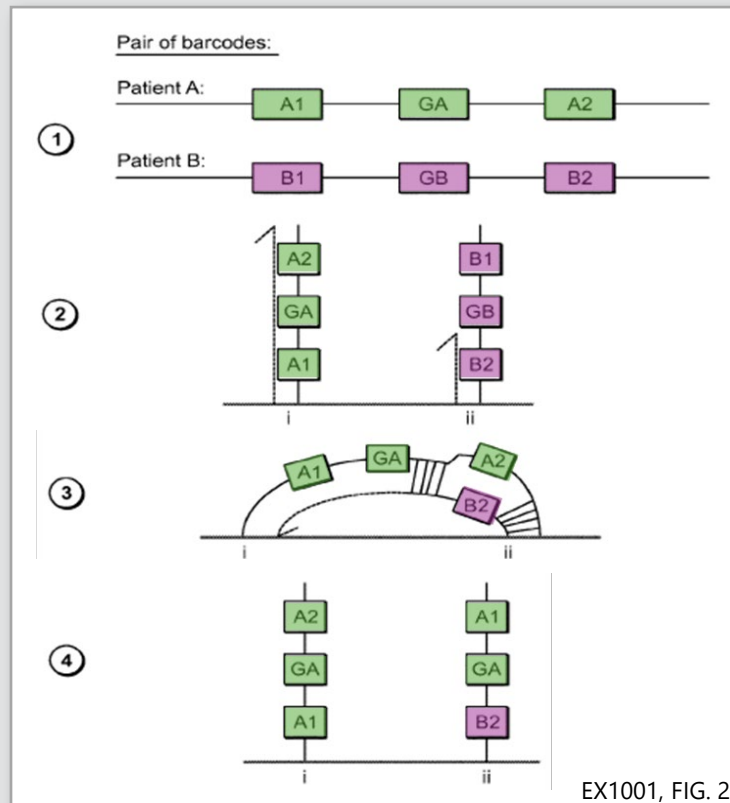
A method for reducing [**error from** *sequences of chimeric molecules created during cluster amplification and made from template nucleic acids derived from different samples*] introduced during sequencing workflow, the method comprising the steps of

...

discarding sequence reads obtained in said sequencing step that contain said combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce [**error from** *sequences of chimeric molecules created during cluster amplification and made from template nucleic acids derived from different samples*].

Patent Owner's Construction Of "Cross-Over Error" Is Supported By The Specification

- Every embodiment in the '852 Patent shows cross-over error caused by overlaps between nucleic acids occurring **during cluster amplification**, and the specification repeatedly describes cross-over error as resulting from those overlaps.



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.

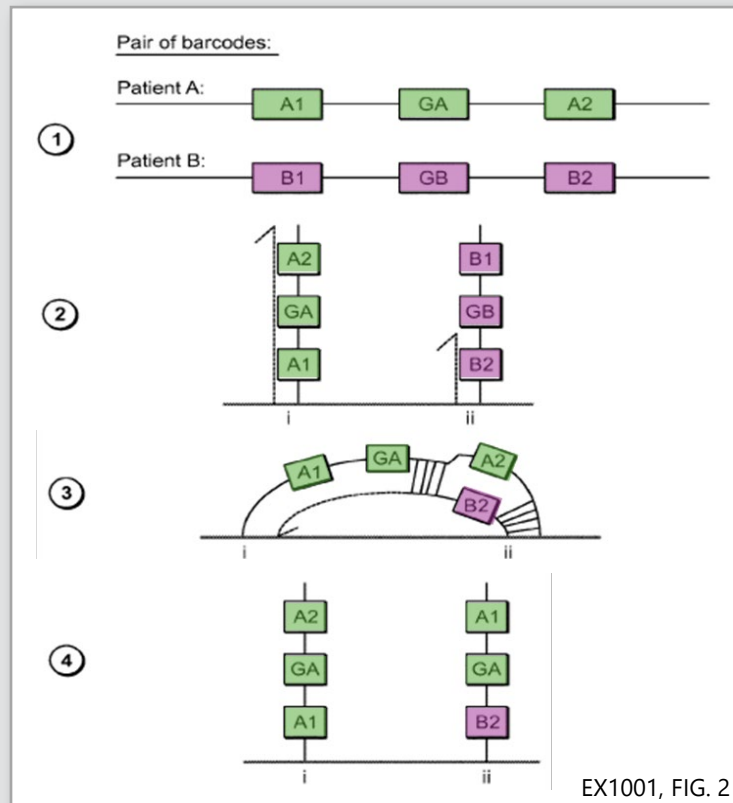
Panel 3 shows an example of improper molecular overlap. After extension, the duplexes are denatured and re-annealed for another amplification cycle. This example shows improper molecular overlap via formation of a heteroduplex between patient A's nucleic acid template and patient B's nucleic acid template.

EX1001, 5:28-46

964 IPR POR, 3-7, 14-18; 964 IPR EX2001, ¶¶26-30, 41-49

Patent Owner's Construction Of "Cross-Over Error" Is Supported By The Specification

- Every embodiment in the '852 Patent shows cross-over error caused by overlaps between nucleic acids occurring **during cluster amplification**, and the specification repeatedly describes cross-over error as resulting from those overlaps.



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.

EX1001, 5:59-6:6

Petitioner's Construction Improperly Removes "Error"

Petitioner's Construction

"sequences of chimeric molecules made from template nucleic acids derived from different samples"

964 IPR Pet., 23

- Petitioner's construction would require "reducing sequences" themselves.
- The error that is reduced in the '852 Patent is **not** the formation of chimeric molecules, but the incorrect identification of sequences based on those chimeras.
- The '852 Patent provides methods to identify the sequences and discard them to reduce error rather than a method to prevent chimeric molecules from forming.

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.

EX1001, 5:59-6:6

964 IPR POR, 13-14; 964 IPR Sur-Reply, 6-7; 964 IPR EX2001, ¶¶24-25, 28

Petitioner's Construction Covering Error From Chimeras Formed At Any Point During Sequencing Is Inconsistent With The Claims And Specification

- **Petitioner argues** that the claimed "cross-over error" can be introduced at any point during sequencing. 964 IPR Reply, 3-4.

1. A method for reducing template cross-over error introduced during sequencing workflow, the method comprising the steps of

incorporating at least two members of a plurality of identifier sequences into template nucleic acids obtained from at least two different samples, wherein said two members constitute a distinct pair associated with said template;

combining said nucleic acid templates into a single sample;

amplifying said nucleic acid templates on a surface of a flow cell thereby to form clusters, at least one of which comprises a chimeric sequence comprising a combination of identifier sequences that are different than any of said distinct pairs;

sequencing amplicons obtained in said amplifying step; and

discarding sequence reads obtained in said sequencing step that contain said combination of identifier sequences that are different than any of said distinct pairs, thereby to reduce cross-over error.

EX1001, claim 1

- But even under Petitioner's construction, the chimeras giving rise to "cross-over error" must be made from "template nucleic acids ***derived from different samples.***"
- This can only occur in the "amplifying" step, ***after*** nucleic acids from different samples are combined.
- And the '852 Patent consistently describes chimeric molecules formed during that amplification step.

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).

EX1001, 7:20-23

964 IPR POR, 13-14; 964 IPR Sur-Reply, 2-4; 964 IPR EX2001, ¶¶23-30

Petitioner's Interpretation Of The "Theory Or Mechanism Of Action" Discussed In The Specification For Chimera Formation Is Too Broad

- **Petitioner argues** that the specification "identifies cluster amplification as merely one possible theory for chimera formation." 964 IPR Reply, 4 (citing EX1001, 6:25-29).

Without being limited by any particular theory or mechanism of action, the data in FIG. 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.

EX1001, 6:25-29

- But both experts agree the '852 Patent merely refers to one biochemical "mechanism of action" (i.e, a heteroduplex) for forming chimeras. And every example in the specification is such a chimera forming **during amplification**.
 - Dr. Adalsteinsson explained "although the specification describes a heteroduplex molecule as one potential way that a chimeric molecule can be formed during amplification, other mechanisms can still occur during amplification to create chimeric molecules." 964 IPR EX2001, ¶29.
 - Dr. Spellman agreed that "a heteroduplex is one mechanism for creating a chimeric sequence but not necessarily the only mechanism." EX2002, 99:18-100:10.

964 IPR POR, 17-18.

Only Patent Owner's Construction Stays True To The Invention

- Petitioner's construction could encompass any event occurring between two template molecules of different samples at any time.
- The '852 Patent discusses and addresses the problem of cross-over error from chimeras formed during cluster amplification with its inventive methods.
- It is undisputed that **every example** of cross-over error in the figures and specification arises from chimeras formed during cluster amplification.

***On Demand Machine Corp. v. Ingram Industries, Inc.*, 442 F.3d 1331, 1340 (Fed. Cir. 2006)**



"Claims cannot be of broader scope than the invention that is set forth in the specification."

Disputed Issues – 964 IPR

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- 2 Lack of motivation to combine Parameswaran and Gloor. (Ground 1)**
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- 4 Petitioner does not show that the combination of Parameswaran and Gloor discloses or renders obvious the "discarding" step of claim 1. (Ground 1)
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- 6 Petitioner's arguments for the combination of Parameswaran and Bentley fail for similar reasons as its combination of Parameswaran and Gloor. (Ground 2)

There Is No Motivation To Combine Parameswaran With Gloor

A POSA would not have been motivated to combine Parameswaran and Gloor because:

- (1) Cross-over error arising in flow cell sequencers was not a recognized problem and was not addressed by the prior art.
- (2) Petitioner ignores significant differences between pyrosequencing and flow cell sequencing, including reasons not to combine that a POSA would have appreciated.
- (3) Petitioner ignores the teachings in both Parameswaran and Gloor espousing combinatorial indexing instead of unique dual indexing.

Cross-Over Error Arising In Flow Cell Sequencing Was Not An Appreciated Problem And Was Not Addressed By Parameswaran Or Gloor

- Prior to the '852 Patent, a POSA would not have appreciated that cross-over errors were an issue for flow cell based sequencing as claimed, including in the asserted prior art, as Dr. Adalsteinsson explained. EX2001, ¶¶105-130.
- Parameswaran posits three potential sources for its “mismatched barcode motifs,” but **none** of these address cross-over errors between molecules of two different samples.
 - (1) “Errors during barcode synthesis or sequencing” are errors in the barcodes themselves, not in amplified template nucleic acids (and not chimeric sequences). EX1004, 7; 964 IPR EX2001, ¶¶120-121.
 - (2) “Contamination in PCR primer stock” is not cross-over error because primers are added **before** samples are combined and thus cannot create chimeras from different samples. EX1004, 7; EX2001, ¶¶122-123.
 - (3) “Cross-contamination in the second round of PCR” at best refers to “pipette errors” or “aerosols” **before** samples are combined and thus is not cross-over error. EX1004, 7; 964 IPR EX2001, ¶¶122-123; EX2002, 137:22-139:9.
- Gloor uses **combinatorial** indexes, which provides a method that could not have discovered chimeric molecules resulting from cross-over errors. 964 IPR EX2001, ¶124

964 IPR POR, 27-34; 964 IPR Sur-Reply, 8-15; 964 IPR EX2001 ¶¶105-130.

Additional References Cited In The Petition Do Not Show An Appreciation Of Cross-Over Error Arising In Flow Cell Sequencing In The Prior Art

- **Petitioner argues in the Petition** it was known that “nucleic acids from different samples can anneal together during PCR amplification, creating erroneous ‘chimeric’ combinations.” 964 IPR Pet., 6-7.
- But Petitioner’s supporting evidence is **pre-NGS** papers discussing standard PCR of **single samples**.
 - Lahr: “[c]apturing the full diversity **within a sample**.” EX1017, 5; EX2001, ¶1107.
 - Meyerhans: “PCR mediated recombination” of single samples **in 1990**. EX1018, 1; EX2001, ¶1108.
 - Paabo: PCR recombination of single samples **in 1989**. EX1019, 1; EX2001, ¶1109.
 - Odelberg: PCR recombination of single samples **in 1995**. EX1020, 1; EX2001, ¶1110.
- Where Petitioner does provide NGS references, those references do not address cross-over error, or any chimeras formed during amplification for sequencing.
 - Creer: discusses only chimeras formed during PCR of single samples. EX1034, 10; EX2001, ¶1112.
 - Dai: discusses only potential issues in library preparation of single samples. EX1033, 8-9; EX2001, ¶1113.

964 IPR POR, 29-30; 964 IPR Sur-Reply, 13-15; 964 IPR EX2001 ¶¶1105-115.

Petitioner Cites New References In The Reply That Should Not Be Considered

- **Petitioner cites in the Reply** to a new set of supporting references to argue that chimeras and cross-over error was a known problem. 964 IPR Reply, 7-10 (citing EX1057, EX1060, EX1061, EX1063, EX1065, EX1066, EX1067).
- Petitioner could have—and should have—submitted this evidence with the Petition, and the late-submitted evidence should be disregarded.



***Intelligent Bio-Systems, Inc. v. Illumina Cambridge, Ltd.*, 821 F.3d 1359, 1369 (Fed. Cir. 2016)**

It is of the utmost importance that petitioners in the IPR proceedings adhere to the requirement that the initial petition identify “with particularity” the “evidence that supports the grounds for the challenge to each claim.” 35 U.S.C. § 312(a)(3). “All arguments for the relief requested in a motion must be made in the motion. A reply may only respond to arguments raised in the corresponding opposition or patent owner response.” 37 C.F.R. § 42.23(b).

Petitioner's New References In Reply Still Do Not Show An Appreciation Of Cross-Over Error Arising In Flow Cell Sequencing In The Prior Art

- **Petitioner argues in the Reply** "Patent Owner and Dr. Adalsteinsson failed to consider numerous prior art references disclosing the problem of chimeric sequences in Illumina sequencing." 964 IPR Reply, 7.
- But even setting aside that Petitioner had never submitted the prior art it accuses Patent Owner of "fail[ing] to consider," the new references still do not show an awareness of cross-over error in flow cell sequencing.
 - Campbell: discusses possible "rearrangements" unrelated to chimeras, including "deletions," "duplications," and "inversions." EX1057, 3 (Table 1); *see also* EX2015, 50:6-17, 51:11-53:8, 55:1-56:4.
 - Varela: similar authors as Campbell discussing "**intra**molecular rearrangements," which cannot be chimeras between different nucleic acids. EX1066, 10.
 - Maher: discusses "identical mate pair" artifacts caused by **optical detection**, not chimeras. EX1061, 8; *see also* EX2015, 122:16-123:6, 28:22-29:12, 30:16-20, 124:17-125:10.
 - Whiteford: discusses "mixed cluster" artifacts caused by **optical detection**, not chimeras. EX1067, 3; *see also* EX2015, 26:2-27:3, 30:21-31:5, 46:6-48:11.
 - Gorzer: discusses initial PCR of **single samples**. EX1060, 1.
 - Smyth: discusses PCR of **single samples**. EX1063, 1.

964 IPR Sur-Reply, 8-15.

The Later Acknowledgements Of Cross-Over Issues On Illumina Sequencers Highlights That They Were Not Known Prior To The '852 Patent

- The realization and acknowledgement of cross-over on Illumina sequencers—including by Illumina itself—further highlights that it was not a know or obvious issue prior to the '852 Patent invention.
- After the '852 Patent, Kircher et al. published the first journal article that actually described a realization that issues from cross-over errors were hampering Illumina sequencing. *See* EX2004.
 - Dr. Adalsteinsson explains that “the industry did not appreciate the crossover error caused by chimeric molecules formed during cluster amplification” prior to that publication. 964 IPR EX2001, ¶143.
- It was not **until 2018** that Illumina addressed the problem and recommended a UDI approach, explaining “[t]he unique dual indexes eliminate hopped reads from downstream analysis, as unexpected combinations are assigned as undetermined and removed from the data,” consistent with the '852 Patent approach. EX2003, 3.
 - Dr. Adalsteinsson explains that prior to that acknowledgement, Illumina had continuously “advertised the use of **single indexes** with its sequencing systems”—the same type of indexes that the '852 aimed to improved by providing a dual-indexing approach. 964 IPR EX2001, ¶128 (citing EX2006, EX2007).

964 IPR POR, 3-4, 7, 35; 964 IPR Sur-Reply, 14-15; 964 IPR EX2001, ¶¶41-44, 128-129.

Petitioner Ignores Significant Differences Between Pyrosequencing And Flow Cell Sequencing, And Reasons Not To Combine

- **Petitioner argues** that a POSA would have combined Parameswaran and Gloor, but fails to sufficiently address the distinctions between the disparate approaches in the references.
- But Parameswaran noted that “**pyrosequencing** is currently limited in complexity (number of samples sequenced in parallel), and in capacity (number of sequences obtained per sample),” and created a “pyrosequencing-tailored” approach for that specific issue. EX1004, Abstract.
- Gloor, a few years after Parameswaran, used Illumina sequencers and explained that with the newer technology was able to sequence **more samples** with “an **exceedingly low** error rate.” EX1005, 2.
 - Gloor also characterized pyrosequencing methods as “**often unsuitable** for reads generated by Illumina sequencing” like the methods used in Gloor. EX1005, 2.
- Dr. Adalsteinsson explained why, in view of those teachings, a POSA would not have viewed Parameswaran’s barcoding methods as having utility in Gloor’s Illumina sequencing in view of those differences and because Illumina sequencers did not have the same needs of pyrosequencing. 964 IPR EX2001, ¶¶91-94.

964 IPR POR, 21-23; 964 IPR EX2001, ¶¶91-94.

Both Parameswaran And Gloor Espouse Using CDI Approaches, Undermining Petitioner's Obviousness Assertions

- **Petitioner argues** that a POSA would have found it straightforward and obvious to apply Parameswaran's UDI approach in Gloor's flow cell sequencing to identify chimeric sequences. 964 IPR Pet., 36-38.
- But Parameswaran actually encouraged using CDIs, **not UDIs**, noting the greater capacity it could provide with "the potential utility of combinations of forward and reverse barcodes to generate up to 48^2 (= 2304) combination barcodes for this set of sequences." EX1004, 8; *see also* EX2002, 147:9-148:3.
- And Gloor similarly highlighted that increased benefit from CDIs, explaining that in its approach "the combination of sequence tags at each end of the overlapped reads **allowed us to use a small number of primers to uniquely tag a large number of samples**." EX1005, 2.
- Dr. Adalsteinsson explained why, in view of those teachings, "[b]ecause Parameswaran identified a low misassignment rate and the utility of combinatorial tagging, and Gloor used CDI and confirmed an exceedingly low error rate, **cost and efficiency would have led a POSA away from using far more tagged primers to sequence fewer samples** [in UDI]." 964 IPR EX2001, ¶97.

964 IPR POR, 23-24; 964 IPR EX2001, ¶¶95-97.

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Petitioner Must Show A Reasonable Expectation Of Success To Meet The Claimed Invention



Intelligent Bio-Systems, Inc. v. Illumina Cambridge, Ltd., 821 F.3d 1359, 1367 (Fed. Cir. 2016)

“The reasonable expectation of success requirement refers to the likelihood of success in combining references to ***meet the limitations of the claimed invention.***”

- Here, the “limitations of the claimed invention” require that a POSA have a reasonable expectation of success in combining the prior art to achieve “discarding sequence reads ... ***thereby to reduce crossover error***” as claimed in independent Claim 1.
 - Dr. Spellman agrees. EX2015, 132:20-133:5.
- Petitioner fails to meet its burden to show a reasonable expectation of success in reducing crossover error, and its obviousness challenge fails for this independent reason.

Petitioner Does Not Show A Reasonable Expectation Of Successfully Reducing Cross-Over Error In Combining The Prior Art

- **Petitioner argues** that a POSA would have a reasonable expectation of success because:
 - (1) Performing Parameswaran's UDI method with Gloor's sequencers would require simply swapping Roche 454 adapters for Illumina-specific adapters (964 IPR Pet., 42-44);
 - (2) Read lengths on Illumina sequencers were appropriate to sequence the nucleic acids in Parameswaran (964 IPR Pet., 44); and
 - (3) Parameswaran's UDI method would reduce errors on Illumina sequencers (964 IPR Pet., 44-45).
- But Petitioner's assertions in the Petition do not specifically address why a POSA would have had a reasonable expectation of **successfully reducing cross-over error** by discarding sequence reads, as claimed.
 - Dr. Adalsteinsson explains that a POSA could not have reasonably expected such success because the prevalence of cross-over error was unknown, either on pyrosequencing machines or on flow cell sequencing machines. 964 IPR EX2001, ¶¶133-134.
- As discussed in connection with motivation to combine, the prevalence of cross-over error on flow cell sequencers was **unknown** prior to the '852 Patent, and a POSA could not have had a reasonable expectation of successfully reducing something that the POSA did not know was present in the first place.

964 IPR Pet., 41-45; 964 IPR Reply, 23-24; 964 IPR POR, 39-41; 964 IPR Sur-Reply, 20-21.

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Petitioner Originally Argued Both Parameswaran And Gloor Disclose The Claimed “Discarding” Step

- Petitioner argued in the Petition that:
 - (1) “Parameswaran discloses claim 1[f] [the “discarding” step]” because it “**distinguished** sequences obtained in the sequencing step that contained combinations of identifier sequences that were different than any of assigned combinations of identifier sequences; (964 IPR Pet., 34-35) and
 - (2) “Gloor also identified and discarded chimeric sequences” because it used a “strict requirement for proper sequence tag and primer sequences” (964 IPR Pet., 35-36).
- Even if Parameswaran “distinguished” its mismatched identifier sequences, it did not **discard** them.
 - Dr. Adalsteinsson explains that “Parameswaran still **kept sequences with incorrect barcode pairs** as long as they contained at least one sequence with a match to one of the two expected barcode motifs.” 964 IPR EX2001, ¶140 (citing EX1004, 8).
 - Indeed, Parameswaran states that it included “all sequences with **a match to one of the two expected motifs**” in its analysis set. EX1004, 8.
- Gloor uses a CDI method that cannot identify mismatched barcodes, much less discard them.
 - Petitioner admitted that Gloor’s “[n]on-unique dual-indexing cannot necessarily identify ‘cross-over’ errors without additional data processing.” 964 IPR Pet., 8.

964 IPR POR, 42-44; 964 IPR Sur-Reply, 21-23; 964 IPR EX2001, ¶¶139-148.

Petitioner Shifts To Only Obviousness In Reply, But Still Fails To Show That The “Discarding” Step Would Have Been Obvious

- **Petitioner argues in reply** that “discarding” chimeric sequences would have been obvious. 964 IPR Reply, 13.
- Petitioner offers new arguments that Parameswaran’s reference to “unexpected” motifs means those motifs are discarded. 964 IPR Reply, 13-15.
 - Parameswaran provides no such explanation and distinguishes sequences having “unexpected” motifs (having one of two expected barcodes) from sequences having zero expected motifs in its data tables. EX1004, 7 (Table 3).
 - And Parameswaran explicitly states that only sequences with “no perfect mismatches” are “discarded,” while “all sequences with a match to **one of the two expected** barcodes” are used in analysis. EX1004, 8; 964 IPR EX2001, ¶¶139-144.
- Petitioner offers new arguments that Parameswaran’s “grayed out” cells in data spreadsheets means the sequences in those cells are discarded. 964 IPR Reply, 13-15.
 - But Dr. Spellman admitted it is the four blank cells in the bottom right corner of Parameswaran’s spreadsheets—**not** the grayed cells—that correspond to sequences that Parameswaran explains were discarded. EX2015, 102:3-12, 102:20-103:9, 103:22-104:19.

964 IPR Sur-Reply, 21-23.

Disputed Issues – 964 IPR

- 1 The intrinsic evidence confirms PO's construction of the "cross-over error" term.
- 2 Lack of motivation to combine Parameswaran and Gloor. (Ground 1)
- 3 Lack of reasonable expectation of success for the combination of Parameswaran and Gloor. (Ground 1)
- 4 Petitioner does not show that the combination of Parameswaran and Gloor discloses or renders obvious the "discarding" step of claim 1. (Ground 1)
- 5** Petitioner does not establish Gloor as prior art. (Ground 1)
- 6 Petitioner's arguments for the combination of Parameswaran and Bentley fail for similar reasons as its combination of Parameswaran and Gloor. (Ground 2)

Petitioner Argues That Gloor Was Publicly Available As Of July 29, 2010

Support Relied On In Petition

Petitioner relied in the Petition on a declaration from Dr. Gloor containing:

- (1) Emails between Dr. Gloor and arXiv.org discussing when Gloor was submitted;
- (2) Dr. Gloor's alleged recollection of a poster describing the findings of Gloor at a conference.

964 IPR Pet., 12; 965 IPR Pet., 11; EX1002.

Support Relied On In Reply

Petitioner relied on new evidence submitted with the Reply:

- (1) A second declaration from Dr. Gloor containing with arXiv.org discussing when Gloor was submitted;
- (2) Archived webpages discussing arXiv.org's submission policies;
- (3) A present-day search of a forum allegedly discussing Gloor;
- (4) A present-day search of Gloor on arXiv.org.

964 IPR Reply, 24–28; 965 IPR Reply, 26–29; EX1046–EX1052 .

The Board Encouraged Petitioner To Develop The Record And Provided Exemplary Sufficient Evidence to Satisfy Petitioner's Burden

In its decision granting Institution, the Board explained that Petitioner only needed to show that "Gloor qualifies as prior art by 'a reasonable likelihood'" but "encourage[d] the parties to fully develop the record during trial to facilitate the ultimate resolution" of Gloor's public availability under the preponderance of the evidence standard.

964 IPR Paper 7, 11–13; 965 IPR Paper 7, 7–10.

The Board later explained that (1) **"testimony"** regarding the internet archive service known as the Wayback Machine" can determine public accessibility and (2) suggested that the **"alleged email arXiv.org sent to subscribers on July 29, 2010,"** announcing the publication of Gloor would constitute competent evidence" to show Gloor's public accessibility.

Paper 16, 5–6 (citing *Murray & Poole Enters. LTD v. Institut De Cardiologie De Montreal*, IPR2023-01064, Paper 49 (PTAB Jan. 14, 2025)).

Petitioner Failed To Provide Competent Evidence That Could Establish That Gloor Was Publicly Available As Of July 29, 2010

- Petitioner did not provide any of the types of evidence the Board has held in the past to be sufficient for establishing a publication's availability, including examples explicitly noted by the Board in these IPRs.
- For example, potentially competent evidence that Petitioner did not provide here include:
 - Petitioner did not provide any archived webpages of the allegedly publicly available link;
 - Petitioner did not provide any metadata associated with the alleged publication (e.g., log data) from a custodian with firsthand knowledge of such data; and
 - Petitioner did not provide any declaration from a librarian or from any custodian at arXiv.org (which allegedly published Gloor) that could provide non-hearsay evidence to establish relevant facts.
- Petitioner's reliance instead on secondhand allegations from Dr. Gloor cannot establish the actual publishing practices of arXiv.org or establish that the Gloor article at issue here was published according to such practices.

Petitioner's Cited Authority Is Inapposite

Petitioner cites *Align Tech*, as support that general evidence of arXiv.org's publication policy at the time of the submission is sufficient to establish the publication date.

964 IPR Reply, 25–26; 965 IPR Reply, 27.

However, the Board in *Align Tech* also considered (1) **archived webpages** containing the links to the arXiv.org publication and (2) **corroborative metadata** associated with the publication link.

Align Tech., Inc. V. Dental Monitoring SAS, IPR 2023-01369, Paper 42 at 31–32.

"The public availability of Maninis to interested persons prior to July 21, 2017, is also corroborated by archived webpages of both arXiv.org and the authors of Maninis. As of **May 3, 2017**, the university website featuring the author's Convolutional Oriented Boundaries software included a link (to <https://arxiv.org/abs/1701.04658>) **for downloading a PDF copy of Maninis from arXiv.org. EX1024 (archived May 3, 2017)**. Additionally, as of **July 8, 2017**, the arXiv.org author search webpage for PrVanof. Luc Gool, the last author listed on Maninis, listed Maninis as one of his recent papers that was available on arXiv.org, **providing links to both the abstract page** (<https://arxiv.org/abs/1701.04658>) **and a link to directly download Maninis** (<https://arxiv.org/pdf/1701.04658>). EX1025 (**archived July 8, 2017**)."

Align Tech., IPR2023-01369, Paper 42 at 31–32 (quoting Ex. 1002).

"Petitioner also produces an additional declaration by Dr. Foroosh attesting that he had '**reviewed metadata contained in a copy of Maninis downloaded from the download link (https://arxiv.org/pdf/1701.04658)**' that 'indicates that the document was created and last modified on April 30, 2017.' Ex. 1029 ¶ 3, Ex. 1002 ¶ 66. Further, Dr. Foroosh indicates that he had downloaded a copy of the Maninis PDF file (Ex. 1007) from the link reproduced above."

Align Tech., IPR2023-01369, Paper 42 at 32.

Petitioner's Use of Archived Webpages Is Insufficient

The Board highlighted *Murray* as an exemplary use of the Wayback Machine to establish public accessibility. In *Murray*, an Internet Archive custodian testified that the Wayback Machine was used to retrieve **archived webpages of the online publication in question**. However, Petitioner's use of the Wayback Machine is limited to arXiv.org's publication policy and does not contain any archived webpages of the online publication.

Paper 16 at 5; 964 IPR Reply, 25–26; 965 IPR Reply, 26–27.

"Dr. Mullins, the second librarian, confirms that Exhibit 1006 – the COLCOT protocol – was publicly accessible by March 2017. In the declaration Dr. Mullins explains that '[t]he Internet Archive is a digital archive/library that began in 1996 to 'scrape' publicly accessible webpages.... It is more or less a 'snapshot' of the website on that date, further information is available at this URL: <https://archive.org/about/>.' Dr. Mullins testifies 'that COLCOT, Exhibit 1006, is an authentic document, and was publicly accessible and documented by the evidence in Attachment A-1, Wayback Machine, October 20, 2017.'"

Murray & Poole Enters. LTD v. Institut De Cardiologie De Montreal, IPR2023-01064, Paper 49 at 22 (PTAB Jan. 14, 2025).

Disputed Issues – 964 IPR

- 1 The intrinsic evidence confirms PO's construction of the "cross-over error" term.
- 2 Lack of motivation to combine Parameswaran and Gloor. (Ground 1)
- 3 Lack of reasonable expectation of success for the combination of Parameswaran and Gloor. (Ground 1)
- 4 Petitioner does not show that the combination of Parameswaran and Gloor discloses or renders obvious the "discarding" step of claim 1. (Ground 1)
- 5 Petitioner does not establish Gloor as prior art. (Ground 1)
- 6** Petitioner's arguments for the combination of Parameswaran and Bentley fail for similar reasons as its combination of Parameswaran and Gloor. (Ground 2)

Petitioner's Obviousness Arguments For Its Ground 2 Combination Tracks Its Arguments For Ground 1, And Fail For The Same Reasons

- Petitioner offers the same deficient reasons for combining Parameswaran with Bentley as it offers for Parameswaran and Gloor, despite Bentley not even disclosing any multiplexing or sample pooling in its sequencing. POR, 51-52; 964 IPR EX2001, ¶¶149-150.
- Petitioner offers the same deficient reasons for reasonable expectation of success in Parameswaran with Bentley as it offers for Parameswaran and Gloor, but still does not show any expectation of **reducing cross-over error** as claimed. POR, 52.
- Because Bentley does not disclose multiplexing, sample pooling, chimeras, or discarding sequence reads, Petitioner relies only on Parameswaran for the “discarding” step, but merely repeats its deficient assertions regarding Parameswaran as it offers for Ground 1. POR, 53; 964 IPR EX2001, ¶152.

964 IPR POR, 51-53; 964 IPR Sur-Reply, 26-27; 964 IPR EX2001, ¶¶149-152.

Additional Disputed Issues – 965 IPR

- 1 Lack of motivation to combine Petitioner's prior art. (Grounds 3-5)
- 2 Petitioner fails to show that the prior art discloses features of dependent claims.

Additional Disputed Issues – 965 IPR

- 1** Lack of motivation to combine Petitioner's prior art. (Grounds 3-5)
- 2** Petitioner fails to show that the prior art discloses features of dependent claims.

Petitioner Fails to Show a POSA Would Have Been Motivated to Combine Parameswaran and Gloor

For the similar three reasons as discussed for the '852 Patent, a POSA would not have been motivated to combine Parameswaran and Gloor to arrive at the claimed invention of the '200 Patent because:

- (1) The industry was not yet aware of chimeric sequences arising in Illumina sequencers and Parameswaran's methods did not address the issue of template cross-over error giving rise to chimeric sequences.
- (2) A POSA would have appreciated the significant differences between pyrosequencing and Illumina sequencing and the downsides of combining the two methods.
- (3) A POSA would have appreciated the costs associated with pursuing Parameswaran's unique dual indexing method over Gloor's method of combinatorial indexing.

Petitioner Fails to Show That A POSA Would Have Been Motivated to Combine Parameswaran and Binladen

A POSA would not have been motivated to combine Parameswaran and Binladen because:

- (1) Neither Parameswaran nor Binladen analyze ***both identifiers*** for the purpose of excluding sequences containing only one identifier or an incorrect pair of identifiers.
- (2) A POSA would not look to incorporate Binladen's barcodes, which were too short for massively parallel runs with many sample libraries, into Parameswaran's multiplexing methods.
- (3) Likewise, Petitioner's proposal to use Parameswaran's longer barcodes to improve Binladen is incompatible with Binladen's reasons for using short barcodes in the first place.

Petitioner Fails to Show That A POSA Would Have Been Motivated to Combine Parameswaran with Binladen and Bentley

A POSA further would not have been motivated to combine Parameswaran with Binladen and Bentley for the same reasons a POSA would not have been motivated to combine Parameswaran with Gloor or Parameswaran with Binladen:

- (1) A POSA would not look to incorporate Parameswaran's method on Illumina sequencers.
- (2) A POSA would not look to incorporate Binladen's barcodes with Parameswaran's method or vice versa.
- (3) None of Parameswaran, Binladen, or Bentley disclose excluding sequences containing only one identifier or an incorrect pair of identifiers – Bentley does not even disclose multiplexing or sample pooling.

Additional Disputed Issues – 965 IPR

- 1 Lack of motivation to combine Petitioner's prior art. (Grounds 3-5)
- 2 Petitioner fails to show that the prior art discloses features of dependent claims.

Gloor Does Not Disclose Claim 5

5. The method of claim 4, wherein the barcode sequences are designed to have no homopolymer regions.

EX1001, claim 5

- Gloor contains no discussion of homopolymer regions or designing barcodes to avoid them.
- Petitioner argues that Gloor's incidental lack of homopolymer regions is sufficient.
- But the claim language requires more than incidental results.

***In re Giannelli*, 739 F.3d 1375, 1379-80 (Fed. Cir. 2014)**



“the mere capability [of performing a function] is not the inquiry that the Board should have made”

965 IPR POR, 15-16; 965 IPR Reply, 5; 965 IPR Sur-Reply, 6-7.

Parameswaran Alone And/Or In Combination With Gloor, Binladen, Or Bentley Does Not Disclose Or Render Obvious Claim 5

5. The method of claim 4, wherein the barcode sequences are designed to have **no homopolymer regions**.

EX1001, claim 5



Dr. Spellman
Petitioner's Expert

Q. So as it's referred to in the Molecular Loop patents, for homopolymers, ***there are two homopolymers in every barcode in Parameswaran, true?***

A. ***That is correct.***

EX2002, 67:3-18

- Parameswaran specifically designed each of its barcodes to ***include homopolymer regions***.
- Petitioner argues that Binladen's incidental lack of homopolymer regions is sufficient, but the claim language requires intent.

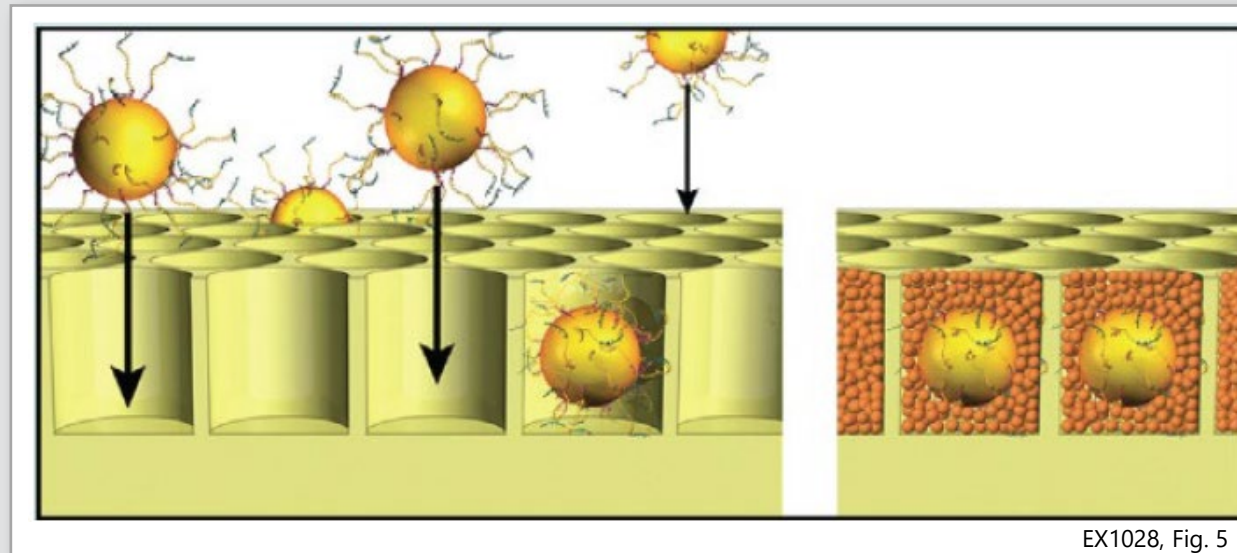
965 IPR POR, 25-26, 46-47, 49-51, 54; 965 IPR Sur-Reply, 11-12, 25, 26; EX2015, 111:14-113:11.

Parameswaran Alone And/Or In Combination With Binladen Or Bentley Does Not Disclose Or Render Obvious Claim 6

6. The method of claim 1, wherein the nucleic acid analyte of interest is on the surface of a flow cell.

EX1001, claim 6

- Parameswaran, Binladen, and Bentley each use pyrosequencing.
- Petitioner does not dispute that the analyte of interest is suspended above the surface of the flow cell in pyrosequencing.



EX1028, Fig. 5

965 IPR POR, 26-27, 51-52; 965 IPR Sur-Reply, 11-12, 26-27; 965 IPR EX1003, ¶190.