



Patent Owner 10x Genomics, Inc.'s Demonstrative Slides

Parse Biosciences, Inc.

v.

10x Genomics, Inc.

IPR2023-00876

Inter Partes Review of U.S. Patent No. 10,155,981

Patent Trial and Appeal Board
United States Patent and Trademark Office
July 24, 2024



Table Of Abbreviations

'981 Patent	EX1001, U.S. Patent No. 10,155,981
Pet.	Paper 1, Petition for <i>Inter Partes</i> Review
ID.	Paper 7, Granting Institution of <i>Inter Partes</i> Review
POR.	Paper 13, Patent Owner's Response
Rep.	Paper 22, Petitioner's Reply to Patent Owner's Response
SurRep.	Paper 26, Patent Owner's Sur-Reply
STRT	Single-cell Tagged Reverse Transcription
NGS	Next Generation Sequencing

* Unless stated, statutory and regulatory citations are to 35 U.S.C. or 37 C.F.R., as context indicates, internal citations omitted, and emphases/annotations are added.

Illustrative '981 Claims

1. A method of analyzing nucleic acids from a plurality of single cells, the method comprising:
 - (a) providing a sample comprising a plurality of single cells, wherein each single cell of the plurality of single cells comprises a plurality of sample polynucleotides;
 - (b) generating a plurality of tagged polynucleotides from the plurality of sample polynucleotides, wherein each tagged polynucleotide comprises:
 - (i) a sequence from a sample polynucleotide of the plurality of sample polynucleotides; and
 - (ii) a multiplex identifier (MID) sequence comprising:
 - I. a first tag sequence associated with the single cell from which the sample polynucleotide is derived, wherein the first tag sequence is a different sequence for different single cells in the plurality of single cells; and
 - II. a second tag sequence distinguishing the sample polynucleotide from other sample polynucleotides derived from the same single cell;
 - (c) sequencing the plurality of tagged polynucleotides to obtain a plurality of identified polynucleotide sequences;
 - (d) using the first tag sequence to correlate the identified polynucleotide sequence with the single cell from which the identified polynucleotide sequence is derived; and
 - (e) using the second tag sequence to correlate the identified polynucleotide sequence with the sample polynucleotide from which the identified polynucleotide sequence is derived.
5. The method of claim 1, wherein the tagged polynucleotides are generated through at least one ligation reaction.

Table Of Contents

Linnarsson’s STRT Method	5
McCloskey Does Not Disclose Single Cell Analysis	20
Ground 1: Petitioner’s Failed Argument That Linnarsson Discloses Sequencing the Entire Polynucleotide	24
Ground 1: Petitioner’s Combination Fails to Satisfy Its Argued Motivation to Combine Linnarsson and McCloskey and Reasonable Expectation of Success	34
Ground 1: Petitioner’s First Tag Theory Is Unclear	44
Ground 2: Litigation Theory Fails To Establish a Motivation to Combine, and Adding Litigation to Petitioner’s Proposed Combination Fails to Meet the Limitations of Claim 5	46
Petitioner’s Analogous Art Analysis Is Deficient	64
Petitioner Failed to Establish McCloskey Is Prior Art	66
Patent Owner’s Motion to Exclude	69
Petitioner’s Motion to Exclude	73
Petitioner’s Reply Should Be Ignored Based on the Extent of Improper Out-of-Scope Reply Arguments	80
Dr. Cooper’s Testimony Should Be Given Little to No Weight	94

Linnarsson's STRT Method



Linnarsson's Disclosures Are All Directed to Describing the "STRT" Method, Which Uses Fragmentation and Capture with Biotin

- Linnarsson's disclosures **all** relate to the Single-cell Tagged Reverse Transcription ("STRT") method. POR.7; SurRep.9-14; EX1003 (Linnarsson), 5:10-11, 23:1-4, 37:3 1-32, Fig.11, Examples I-II; EX2020 (Cooper Reply Dep. Tr.), 96:12-97:11, 118:5-8.
- The purpose of Linnarsson's method is to generate a "cDNA library."
POR.7; SurRep.12; Pet.8-9 ("Linnarsson discloses a method for preparing a cDNA library"); EX1003 (Linnarsson), 1 (Abstract); EX1002 (Cooper Decl.), ¶108.
- Linnarsson defines "cDNA Library" as "a collection of cloned complementary DNA (cDNA) **fragments**."
EX1003 (Linnarsson), 12:4-8; EX2018 (Cooper Dep. Tr.), 41:10-44:22; POR.23; SurRep.12.

And Dr. Cooper admits the cDNA *must be fragmented* for the next-generation sequencing ("NGS") Linnarsson discloses:

"[A]t some point, you need to fragment to get them -- to make them suitable for the particular sequencer machine that you're using."

EX1003 (Linnarsson), 6:9-12; EX2018 (Cooper Dep. Tr.), 32:7-14, 42:11-43:3, 97:17-101:22, 111:8-113:16; POR.12, 23; SurRep.11-12; see Slide 18.

Linnarsson's Disclosures Are All Directed to Describing the "STRT" Method, Which Uses Fragmentation and Capture with Biotin

- Linnarsson's fragment and capture STRT method **requires biotin** for capture.

EX2013 (Quackenbush Decl.), ¶¶90-91; POR.55-56 n.21; SurRep.9-14, 19.

- Linnarsson consistently discloses fragmentation and capture with biotin, including in both of its Examples (Examples I and II) and in Figure 11.

See Slides 9-17; EX2013 (Quackenbush Decl.), ¶¶90-91; EX1003 (Linnarsson), Fig.11, 5:28-6:12; 23:1-37:32; EX2020, (Cooper Reply Dep. Tr.), 94:3-7; POR.55-56, 55 n.21; SurRep.3, 9-14, 19.

- Biotin is critical in Linnarsson's method, as it **allows for tagged polynucleotides to be isolated from untagged polynucleotides.**

See Slide 51; POR.23-25; EX2013 (Quackenbush Dec.), ¶¶90-91; POR.55-56, 55 n.21; SurRep.9-14, 19.

- Without such purification, tagged and untagged polynucleotides would be sequenced and the resulting information would be muddled. SurRep.16; POR.55-56, 55 n.21; EX2013 (Quackenbush Decl.), ¶90.
- As Dr. Quackenbush explains, "*Linnarsson discloses that the biotin captures the tagged fragments and enables their purification. ... Without biotin, all the fragments would be 'washed away.'*" EX2013 (Quackenbush Decl.), ¶90; SurRep.16; Rep.55 n.21.

Petitioner Relies on Figure 11, Which Depicts Generating a cDNA Library Via the STRT Method, Including Fragmentation and Capture with Biotin

- On Reply, Petitioner first argued that Linnarsson’s Figure 11 is not its “sole embodiment” to avoid Linnarsson’s requirements of fragmentation and capture with biotin.
Rep.5-6; SurRep.9-14.
- But the Petition and Dr. Cooper both expressly rely on Figure 11, which requires *fragmentation* and *biotin for capture*.

POR.7; SurRep.14-15; Pet.25-27, Pet.41-42, Pet.45-47; EX1002 (Cooper Decl.), ¶¶110, 113, 117, 139, 143, 153, 154, 164, 166; EX2018 (Cooper Dep. Tr.), 23:15-25:21, 29:14-30:2; EX2020 (Cooper Reply Dep. Tr.), 82:7-84:17.

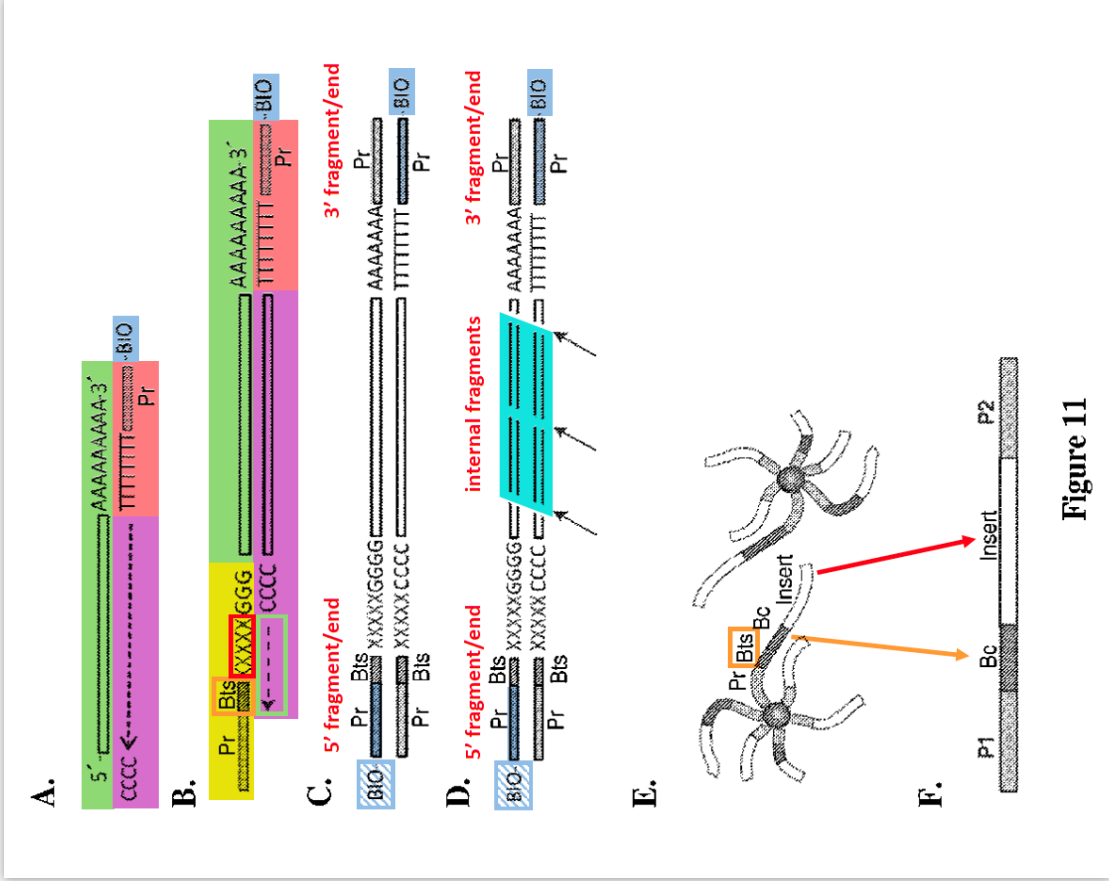


Figure 11

Linnarsson’s Only Two Examples (I and II) Both Employ Fragmentation and Capture With Biotin

- Petitioner now argues Figure 11 uses a series of “steps optionally used in the STRT method,” and that Figures 3 and 4 demonstrate “Linnarsson cannot possibly require fragmentation as in Figure 11” because they do not include biotin or a restriction cut site.

Rep.5-6; EX1059 (Cooper Reply Decl.), ¶¶64-72; SurRep.9-10.
- But this improper new argument is wrong and contradicted by Petitioner’s and Dr. Cooper’s own Petition mapping.

SurRep.9-15.
- In the Petition, Petitioner and Dr. Cooper took disclosures from each of **Figures 2, 3, 4, and 11** and **presented them together as a single embodiment** to argue disclosure of ‘981’s claim limitations.

“As shown in **Figures 2 and 11**, a CDS (boxed in green, below)... As shown in **Figure 4**, Linnarsson discloses that **the** CDS (boxed in green)....”

Diagram showing a DNA sequence with a poly-A tail (AAAAAA) and a primer binding site (Pr). The CDS is boxed in green. A primer (Pr) is shown binding to the 3' end of the CDS. The sequence is labeled 5' and 3'.

EX1003 at Fig. 2

Diagram showing a DNA sequence with a poly-A tail (AAAAAA) and a primer binding site (Pr). The CDS is boxed in green. A primer (Pr) is shown binding to the 3' end of the CDS. The sequence is labeled 5' and 3'.

Id. at Fig. 11

EX1003 at Fig. 4

Diagram showing a DNA sequence with a poly-A tail (AAAAAA) and a primer binding site (Pr). The CDS is boxed in green. A primer (Pr) is shown binding to the 3' end of the CDS. The sequence is labeled 5' and 3'.

Pet. 41-42, 26-29, 42-43 (“As shown in Figure 2 below, a TSO... Figure 11 discloses the same... Figure 3... similarly discloses that the TSO...”), 45-47; SurRep.13-14; EX1002 (Cooper Decl.), ¶¶139-141 (“As demonstrated in Figures 2 and 11, above, the CDS...”), 143 (“The TSO... is shown in Figures 2 and 11”), 153 (“Linnarsson’s Figures 2 and 11, depicted below, show...”), 154-156 (“As can be seen below in Figures 2 and 11”), 163-166; EX2020 (Cooper Reply Decl. Tr.), 82:7-84:17.

Demonstrative Exhibit – Not Evidence

9

Linnarsson's Only Two Examples (I and II) Both Employ Fragmentation and Capture With Biotin

- As Petitioner's Dr. Cooper admits, **Figures 3 and 4 are both introduced as part of Example I.**

Rep.9-10; SurRep.9-14; EX2020 (Cooper Reply Dep. Tr.), 90:9-92:19, 94:8-97:11 & 118:5-13 (acknowledging Figures 3 and 4 are in Example I, but arguing Figures 3 and 4 are "incompatible with a fragmentation and capturing approach" while Example I "discloses fragmentation and capture"); EX1003 (Linnarsson), 23:1-37:32; EX2013 (Quackenbush Decl.), ¶¶36, 89-90.

- And Example I and Example II, in turn, are both fully consistent with Figure 11—**both explicitly require *biotin* and *fragmentation*:**

- Both use a *biotinylated* CDS and a TSO with a restriction enzyme *cut site*,
- Both add a *biotin* to each TSO during PCR amplification,
- Both *fragment* the amplified polynucleotides, and then
- Both sequence just the 5' *fragment*.

Rep.9, n.5; EX2013 (Quackenbush Decl.), ¶¶36, 89; EX1003 (Linnarsson), 23:9-19, 31:3-32:27; EX2018 (Cooper Dep. Tr.), 23:15-25:21, 29:14-30:2; EX2020 (Cooper Reply Dep. Tr.), 96:12-97:11, 118:5-8; EX2101 (Guan), 1; POR.53, 55 n.21; SurRep.9-10.

Linnarsson's Only Two Examples (I and II) Both Employ Fragmentation and Capture With Biotin (*cont'd*)

Example I

- "Single-cell tagged reverse transcription (STRT)"
EX1003 (Linnarsson), 23:2; SurRep.10.
- "The sequence of STRT-T30-BIO (which is a CDS) is: 5'-**BIO**-AAGCAGTGGTATCAACGCAGAGT30VN-3'"
EX1003 (Linnarsson), 23:13-14; POR.53.
- "The sequence of STRT-PCR is: 5'-**BIO**-AAGCAGTGGTATCAACGCAGAGT-3'"
EX1003 (Linnarsson), 25:17-18 (Example I); POR.53.
- "The sample was treated with DNaseI... to generate **double-strand breaks** and **reduce the size**"
EX1003 (Linnarsson), 26:10-11; POR.25.
- "The **fragments** were next **bound to beads** to **capture** 5' and 3' ends... About 10% of the sample was **bound to the beads**...since internal fragments were not biotinylated."
EX1003 (Linnarsson), 26:22-26; POR.12.
- "The **fragments** were released by BtsCI digestion..."
EX1003 (Linnarsson), 27:6-8; POR.23-24

Example II

- "Single-cell tagged reverse transcription (STRT)"
EX1003 (Linnarsson), 32:10; POR.53.
- "STRT-T30-BIO (5'-**BIO**-AAGCAGTGGTATCAACGCAGAGT-3')"
EX1003 (Linnarsson), 32:19-20; POR.53.
- "STRT-PCR primer (5'-**biotin**-AAGCAGTGGTATCAACGCAGAGT-3')"
EX1003 (Linnarsson), 35:8-10; POR.25.
- "Amplified cDNA was **fragmented** by DNase I ...which causes a preference for **double-strand breaks**."
EX1003 (Linnarsson), 35:17-19; POR.25.
- "5' **fragments** containing barcodes and cDNA inserts were **released from the beads** by BtsCI digestion..."
EX1003 (Linnarsson), 35:28-30; POR.23-24.

Figures 3 and 4 Are Both Disclosed as Part of (and Are Fully Consistent With) Example I's Fragmentation and Capture With Biotin

- Petitioner argues Figures 3 and 4 “include neither a **biotin** nor a restriction enzyme cut site.”
Rep.6 (citing EX1059 (Cooper Reply Decl.), ¶¶36-42, 66-68).
- But as part of illustrating Example I's process, which generates a cDNA library with the STRT process's fragmentation and capture approach, Figures 3 and 4 show *nucleotide sequences* for a “typical” TSO and CDS.
SurRep.10; EX1003 (Linnarsson), Figs.3-4.
- Consistent with Example I's STRT disclosures, Figures 3 and 4 include, *inter alia*, a primer (APS) and cell-tag.
SurRep.9-10; EX1003 (Linnarsson), Figs.3-4;



Figure 3

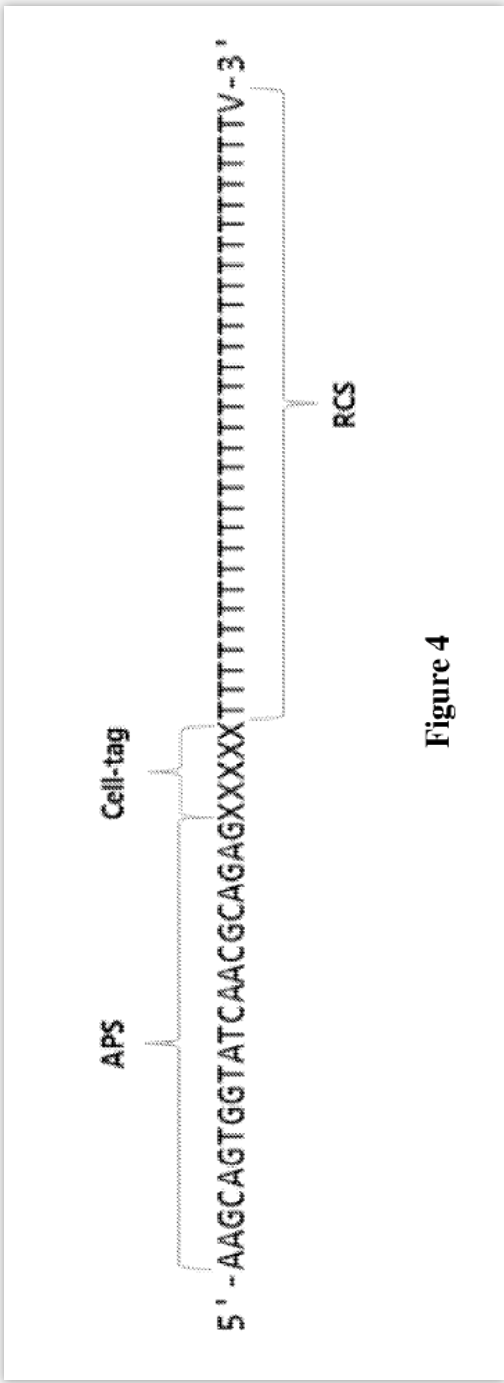


Figure 4

Figures 3 and 4 Are Both Disclosed as Part of (and Are Fully Consistent With) Example 1's Fragmentation and Capture With Biotin (*cont'd*)

- Petitioner argues Figures 3 and 4 “include neither a biotin nor a **restriction enzyme cut site**.”

Rep.6 (citing EX1059 (Cooper Reply Decl.), ¶¶36-42, 66-68).

Dr. Cooper testified that:

“**biotin** (in the case of both the CDS and TSO) and **restriction enzyme cut site** (in the case of the TSO) are **part and parcel of the fragmentation method** used in Figure 11.”

EX1059 (Cooper Reply Decl.), ¶31; see also EX2020 (Cooper Reply Dep. Tr.), 129:2-7; SurRep.9-10.

And, attempting to distinguish them from Figure 11, Dr. Cooper repeatedly opined that Figures 3 and 4 do not contain “any” “**restriction enzyme cut site at all**”:

“Figures 3 and 4 **do not include... a restriction enzyme cut site at all**....”

“Linnarsson’s Figure 3 and Figure 4 show exemplary TSO and CDS that do not contain a biotin and **do not contain any restriction enzyme cut site**.”

E.g., EX1059 (Cooper Reply Decl.), ¶¶118, 66-67, 42 (“The lack of a restriction enzyme cut site... in Figure 3 is critical”); Pap.38, at 4.

Figures 3 and 4 Are Both Disclosed as Part of (and Are Fully Consistent With) Example 1's Fragmentation and Capture With Biotin (*cont'd*)

- But Cooper then **admitted** at deposition that:
 - He **did no investigation** as to whether any cut site other than the one specified in Figure 11 was present, and
 - He **could not dispute that another cut site might be present**, despite his sweeping testimony to the contrary.
Rep.10; SurRep.9-10; EX1003 (Linnarsson), Figs.3-4; EX2101, 1; EX2020 (Cooper Reply Dep. Tr.), 123:11-124:21, 123:11-124:21, 125:5-22, 133:19-137:10.
 - In particular, Dr. Cooper admitted that:
 - “[P]eople of skill at the time of the ‘981 patent *had resources available to identify restriction[] cut sites*.”
EX2020 (Cooper Reply Dep. Tr.), 120:20-121:16; SurRep.9-10
 - He *“did not” “look for any other cut site”* other than “the cut site that is referred to in Figure 11.”
EX2020 (Cooper Reply Dep. Tr.), 120:7-18, 137:7-10; Rep.9-10.
 - He *did not know “one way or the other” whether “GCAGTG” was a restriction enzyme cut site*.
EX2020 (Cooper Reply Dep. Tr.), 123:11-124:16; SurRep.9-10.
 - He *“wouldn’t say one way or another” whether Figure 11 would work with another restriction enzyme* other than BtsCI.
EX2020 (Cooper Reply Dep. Tr.), 127:21-129:7; SurRep.9-10.
 - EX2101 is an article from a well-respected source that states that **“GCAGTG” is a restriction enzyme cut site**.
EX2020 (Cooper Reply Dep. Tr.), 123:6-124:21, 133:19-137:10; SurRep.10-11.

Figures 3 and 4 Are Both Disclosed as Part of (and Are Fully Consistent With) Example 1's Fragmentation and Capture With Biotin (cont'd)

- Dr. Cooper admitted:

- He was familiar with “New England Biolabs” (abbreviated “NEB”).
- That “one of their main products” is “publications and posters that show enzymes and cut sites[.]”

Q. And they're prominent in the field of enzyme restriction sites and so forth?

...

A. They're a company that sells a lot of restriction enzymes, yes.

- EX2101 is from New England Biolabs.
- EX2101 identifies **GCAGTG** as an enzyme restriction site.

Linnarsson (EX1003)



Figure 3

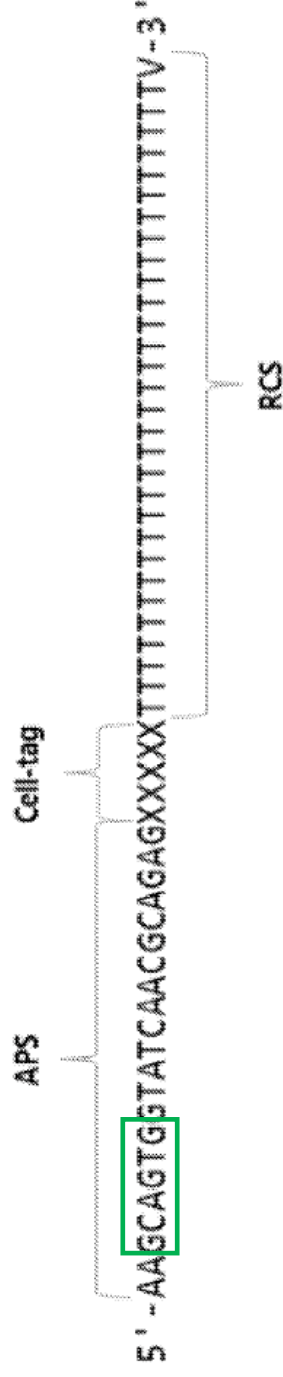


Figure 4

SurRep.10; EX2020 (Cooper Reply Dep. Tr.), 121:2-21, 133:22-134:20.

Figures 3 and 4 Are Both Disclosed as Part of (and Are Fully Consistent With) Example 1’s Fragmentation and Capture With Biotin (cont’d)

- Dr. Cooper admits that EX2101 states that:
 - “The Type IIS restriction endonuclease BtsI recognizes and digests at **CGAGTG (2/0)**”
 - “The cleavage sequence is **GCAGTG (2/0)**, which means that BtsI cuts 2nt” – or nucleotides – “after the recognition sequence on the top strand and right after the recognition sequence on the bottom strand.”

- Q. Does [EX2101] refresh your recollection one way or the other about whether GCAGTG is an enzyme restriction site?

A. Again, I’d have to read through this paper. I don’t -- it’s from New England Biolabs, and that’s what they’re saying, but I -- you know, it’s hard to -- these are new England Biolabs authors in a PLOS One paper; I’d have to do more homework on this. I don’t...

Q. And you didn’t do that before you testified that there were no restriction sites in Figures 3 and 4 right?

A. I did not look at this paper.

EX2020 (Cooper Reply Dep. Tr.), 135:22-136:5, 136:13-137:10; SurRep.10-11.



OPEN ACCESS
Freely available online

Alteration of Sequence Specificity of the Type IIS Restriction Endonuclease BtsI

Shengxi Guan, Aine Blanchard, Penghua Zhang, Zhenyu Zhu*
New England Biolabs, Inc., Ipswich, Massachusetts, United States of America

Abstract

The Type IIS restriction endonuclease BtsI recognizes and digests at GCAGTG(2/0). It comprises two subunits: BtsA and BtsB. The BtsB subunit contains the recognition domain for bottom strand nicking and part of the cleavage sequence, while the BtsA subunit contains the recognition domain for top strand nicking. BtsA alone has no activity unless it mixes with BtsB to reconstitute the BtsI activity. During characterization of the enzyme, we identified a BtsB mutant R119A found to have a different digestion pattern from the wild type BtsI. After characterization, we found that BtsB(R119A) is a novel restriction enzyme with a previously unreported recognition sequence CAGTG(2/0), which is named as BtsI-1. Compared with wild type BtsI, BtsI-1 showed different relative activities in NEB restriction enzyme reaction buffers NEB1, NEB2, NEB3 and NEB4 and less star activity. Similar to the wild type BtsB subunit, the BtsI-1 B subunit alone can act as a bottom nicking enzyme recognizing CAGTG(-/0). This is the first successful case of a specificity change among this restriction endonuclease type.

Citation: Guan S, Blanchard A, Zhang P, Zhu Z (2010) Alteration of Sequence Specificity of the Type IIS Restriction Endonuclease BtsI. PLoS ONE 5(7): e11787. doi:10.1371/journal.pone.0011787

Editor: Fernando Rodriguez-Lima, University Paris Diderot/Paris 7, France

Received: April 2, 2010; **Accepted:** June 30, 2010; **Published:** July 26, 2010

Copyright: © 2010 Guan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: New England Biolabs supported the work. As the authors are all employed by New England Biolabs, the funders were involved in study design, data collection and analysis, decision to publish, and preparation of the manuscript.

Competing Interests: The authors have no competing financial interests. The enzymes and products, BtsI and BtsI-1, which can be commercial products, are already covered by pending patent application. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: zhuzhu@neb.com

Introduction

Restriction-Modification (R-M) systems mostly occur naturally in bacteria and serve as defensive systems against invading DNA, particularly bacteriophages. The restriction endonucleases digest invading DNA while the DNA methyltransferase component(s) (cognate methyltransferase(s)) modifies the host genome and protects the host DNA from restriction endonuclease digestion [1]. There are three groups of restriction endonucleases (REases): Type I, Type II, Type III and Type IV [2]. Among them, Type II REases have been best studied and used as tools in molecular biology, largely due to their well-defined recognition and cleavage sites. So far, more than 3800 Type II REases with 299 distinct specificities have been characterized biochemically or genetically [3]. Among the subdivisions of Type II REases, the Type IIS are unique in having asymmetric recognition sequences [2].

BtsI (Genbank: C8080224.1), a Type IIS REase, was isolated from *Bacillus thuringiensis* (Pan, X.S., Morgan, R. unpublished observation). BtsI REase consists of two subunits BtsA and BtsB, and the modification system also contains two methyltransferases (M1.BtsI and M2.BtsI) [4]. Each methyltransferase modifies one strand of the recognition sequence. BtsB, the large subunit, contains a DNA recognition domain and a catalytic domain that can act naturally as a bottom strand nicking enzyme. The recognition sequence is written as GCAGTG(-/0), which means that BtsB does not cut the top strand and cuts right after the recognition sequence on the bottom strand. BtsA, the small subunit, contains part of the catalytic domain responsible for top strand cleavage. BtsA alone is inactive. Only when BtsA is

combined with BtsB do they form a restriction endonuclease capable of double-strand cleavage (GCAGTG(2/0)) [4]. The cleavage sequence is GCAGTG(2/0), which means that BtsI cuts 2 nt after the recognition sequence on the top strand and right after the recognition sequence on the bottom strand.

Numerous efforts have been made to engineer REases for altered specificity but with rather few successful examples. Several groups reported success in altering the specificity of Type IIG REases. For example, Eco31I (CTGAAG(16/14)) has been engineered to Eco31MI (CTGAC(16/14)) by random mutagenesis followed by challenges with Eco31I (CTGAAG(16/14)) and GnuI (CTGGAC(16/14)) [5]. And (12-15)GAA(NNNNNNTTC(12-13/7)) a Type IIG-like REase was engineered to recognize a new sequence and digestion specificity by domain swapping with another similar Type IIG REase [6]. One major cluster of successful examples is from MndI(TCGAC(20/18) or TCGAC(21/19)-like Type IIG REases. By analyzing the amino acid sequence of a group of closely related REases, specificity can be rationally altered by changing the corresponding amino acid residues in the protein [7]. All of these successful examples are Type IIG REases, in which endonuclease, methylase, and specificity domains are fused into a single polypeptide chain.

Other than Type IIG REases, there are reports of incomplete specificity changes with certain preference for one particular sequence. For example, some mutants of BsoBI change specificity from C/ YCGGG to prefer C/CCGGGG, C/TCGGGG or C/TCGAG[8]. BstYI has been engineered to favor A/GATCT than G/GATCT [9]. HincII (QJ380) shows a stronger preference for GTTATAC and GTTCGAC than wild type enzyme [10]. However, engineering the catalytic domain is relatively easier than engineering the recognition

Figures 3 and 4 Are Both Disclosed as Part of (and Are Fully Consistent With) Example 1's Fragmentation and Capture With Biotin (*cont'd*)

- As Dr. Cooper admits, "a restriction enzyme cut site is part and parcel of the fragmentation method," which is the method used in Linnarsson's Figure 11.

EX2020 (Cooper Reply Dep. Tr.), 123:11-125:4, 129:2-7; Rep.5, SurRep.10-11; EX1059 (Cooper Reply Decl.), ¶31.

Linnarsson (EX1003)



Figure 3

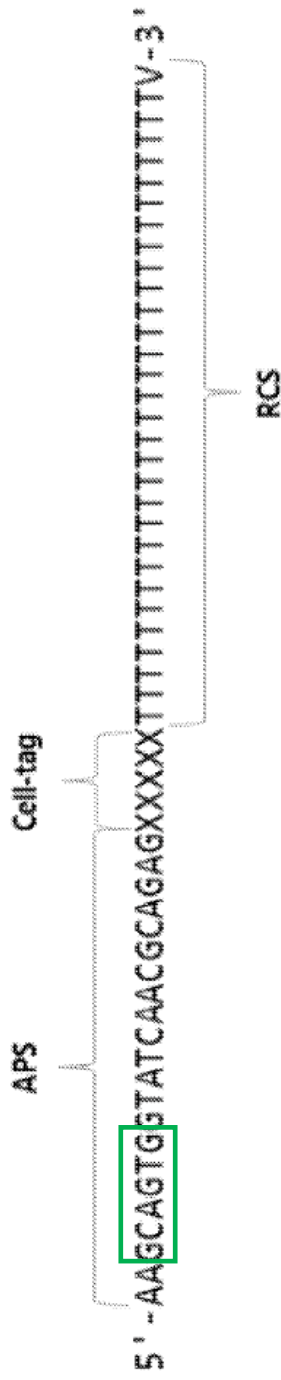


Figure 4

Linnarsson Discloses That Further Fragmenting May Be Needed to Generate the cDNA Library

- Linnarsson’s “cDNA Library” is already fragmented: it is “a collection of cloned complementary DNA (cDNA) **fragments**.”
EX1003 (Linnarsson), 12:4-8; EX2018 (Cooper Dep. Tr.), 41:10-44:22; POR.23; SurRep.11-12 & n.7.
- And as Petitioner’s Dr. Cooper admits, Linnarsson discloses that, after generating the cDNA library, *additional* fragmentation may be required for, *e.g.*, next-generation sequencing (“NGS”) or for shotgun sequencing.

“certainly one could contemplate many fragmentation steps if one wanted to.”

SurRep.11-12; EX1003 (Linnarsson), 22:5-21; EX2020 (Cooper Reply Dep. Tr.), 97:17-103:19, 111:8-113:16.

- For example, Dr. Cooper confirms the cDNA *must be fragmented to a suitable size* for the next-generation sequencing (“NGS”) Linnarsson discloses:

“[A]t some point, **you need to fragment** to get them – to make them suitable for the particular sequencer machine that you’re using.”

EX1003 (Linnarsson), 6:9-12, 12:4-8; EX2018 (Cooper Dep. Tr.), 32:7-14, 42:11-43:3; POR.12, 23; SurRep.12.

The Petition Offered No Modifications to Linnarsson’s Use of Fragmentation and Biotin

- The Petition and Dr. Cooper’s first declaration never acknowledged the **fragmentation** in Linnarsson and included Linnarsson’s **biotin** molecules in their mapping callouts.

Pet.41; EX1002 (Cooper Decl.), ¶¶139-141, 143, 153-156, 163-166; EX2020 (Cooper Reply Dep. Tr.), 76:13-78:20 (admitting he “didn’t discuss the biotin molecule because I didn’t feel it was particularly important”), 82:7-84:17; SurRep.14-15.

- The Petition’s and Dr. Cooper’s only proposed modification to Linnarsson was to add McCloskey’s 7-nucleotide barcode.

“McCloskey discloses that its batch-stamp typically comprises 6 to 8 nucleotides, and its barcode comprises 7 nucleotides. Therefore, a POSA could have conceptually designated the first 6-8 nucleotides of Linnarsson’s cell-tag sequence to identify the single-cell origin of the tagged polynucleotide, and use the next 7 nucleotides to distinguish the tagged polynucleotide from other one from the same single cell.”

Pet.37-59; EX1002 (Cooper Decl.), ¶¶ 192-193; POR.33-34; SurRep.6-8.

- Dr. Cooper later testified at deposition that Figure 11 includes “a series of optional steps” but never asserted any aspect was optional in his opening declaration, and never explained how a modified disclosure **without** such steps would work.
EX2018 (Cooper Dep. Tr.), 22:5-12 (Figure 11 has some of the “key steps”), 23:4-14 (refers to optional steps, but again refers to “key steps”), 25:10-21, 31:1-22; POR.9, 25, 55 n.21.
- Neither the Petition nor Dr. Cooper’s opening declaration suggests eliminating fragmentation or biotin, or explains how Linnarsson’s method would work without either feature.
POR.55-56 n.21; EX1002 (Cooper Decl.), ¶¶110, 113, 117, 139, 143, 153, 154, 164, 166 (relying on Figure 11’s biotinylated CDS; EX2013 (Quackenbush Decl.), ¶¶90-91; SurRep.8-9.
- And this is quite understandable, as Linnarsson’s method consistently requires both fragmentation and biotin.

See Slides 6-17.

McCloskey Does Not Disclose Single Cell Analysis



McCloskey Discloses A Process For Identifying Contamination Across Different Batches

- McCloskey describes a process for identifying contamination between different batches (*e.g.*, between samples from different patients).
POR.15, 30-31; EX1004 (McCloskey), 2-4; EX2013 (Quackenbush Decl.), ¶¶45-47, 62-64.
- McCloskey's method involves lysing the cells and **combining their contents (including the DNA) before tagging** the copies of a particular gene.
POR.16-17 (between samples from different patients); POR.15; EX1004 (McCloskey), 2-4; EX2018 (Cooper Dep. Tr.), 34:19-35:3; EX2013 (Quackenbush Decl.), ¶¶47-48.

McCloskey’s Encoding Oligonucleotide Includes a 7-Nucleotide Barcode

- McCloskey describes adding an “encoding oligonucleotide” to copies of alleles for a particular gene.
POR.15; EX1004 (McCloskey), 2-4; EX2013 (Quackenbush Decl.), ¶46.
- McCloskey’s encoding oligonucleotide includes (1) a batch-stamp, (2) a random barcode, and (3) a genomic target-binding site that targets the specific gene on the chromosomal DNA to be barcoded.
POR.15; EX1004 (McCloskey), 2; EX2013 (Quackenbush Decl.), ¶46.
- McCloskey’s *batch-stamp* identifies the batch sample from which a polynucleotide originates in order to identify contamination, and is 6-8 nucleotides long.
POR.15; EX1004 (McCloskey), 2-4; EX1002 (Cooper Decl.), ¶192; EX2013 (Quackenbush Decl.), ¶46.
- McCloskey’s *barcode* distinguishes between copies of an allele for a particular gene in the sample, and is 7 nucleotides long.
POR.15-16; SurRep.6-8; EX1004 (McCloskey), 2-4; Pet.56 (citing EX1002 (Cooper Decl.), ¶192); EX2013 (Quackenbush Decl.), ¶46.

McCloskey’s Approach Cannot Identify the Cell From Which a Polynucleotide Originates

- Petitioner and Dr. Cooper say McCloskey teaches single cell analysis. Pet.30; EX1002 (Cooper Decl.), ¶199; POR.39-40.
- But McCloskey does **not** disclose analysis of a sample consisting of a plurality of cells where the sample can be analyzed on a cell-by-cell basis. Pet.30; EX1002 (Cooper Decl.), ¶199; EX2013 (Quackenbush Decl.), ¶¶ 62-66, 77-78; POR.28-32, 39-40.

– McCloskey teaches that *each sample uses the same batch-stamp*, regardless of sample size:

“The assigned batch-stamp is registered for a specific sample run on a specific date and is used only once.”

- And McCloskey teaches *pooling all the DNA from a sample prior to tagging*, which makes it impossible to determine from which cell a particular allele originated. POR.32; EX1004 (McCloskey), 2, 5; EX2013 (Quackenbush Decl.), ¶166; EX2018 (Cooper Dep. Tr.), 33:3-35:19; see also EX1002 (Cooper Decl.), ¶176 (“isolation of single cells from a cell population” is the first step of “Single Cell Genomics”).
- McCloskey only discloses the possibility of tagging (for detecting contamination) when the sample itself consists only of a “single cell,” which is not “single cell analysis” as Petitioner argues. POR.28-32; EX1004 (McCloskey), Abstract, 7; EX2013 (Quackenbush Decl.), ¶162-66; EX2018 (Cooper Dep. Tr.), 38:6-39:17.

Ground 1: Petitioner's Failed Argument That Linnarsson Discloses Sequencing the Entire Polynucleotide



Petitioner’s Sequencing Assertions Fail—Linnarsson Does Not Sequence the Entire Tagged Polynucleotide

- The *Petition argued* Linnarsson meets the sequencing limitation because *Linnarsson teaches sequencing the entire tagged polynucleotide*.
 - The Petition and Dr. Cooper equated the “tagged cDNA” to the full-length cDNA molecules generated by reverse transcription. Pet.25, 39-41, 44; EX2013 (citing EX1002 (Cooper Decl.), ¶138); POR.22-25; SurRep.8-9.
 - The *Petition argued* “Linnarsson teaches that after generating *the tagged polynucleotides*, ‘sequencing is performed’ *thereon*.” Pet.44, 56-57; POR.24; SurRep.8-9.
- But *Linnarsson does not teach sequencing the entire polynucleotide*. Pet.44, 56-57; POR.24; EX2013 (Quackenbush Decl.), ¶¶55-58; SurRep.8-16.
 - Petitioner ignored that Linnarsson sequences only the “5’ fragment” and failed to address whether or how sequencing only the “5’ fragment” is sufficient to meet the claim element based on Petitioner’s argument. POR.22-23; SurRep.16-17; EX2018 (Cooper Dep. Tr.), 44:10-44:22; EX2013 (Quackenbush Decl.), ¶¶55-59.
 - Petitioner repeatedly relied on Figure 11 in its mappings, and on Reply Petitioner admits “in Figure 11 of Linnarsson, the CDS is not sequenced.”
Rep.7-8; SurRep.14, 17; EX2013 (Quackenbush Decl.), ¶¶59; Pet.41-42, 45-47 (mapping Fig.11 to [1C], [1D]); EX1002 (Cooper Decl.), ¶¶139-141, 143, 153-156, 163-166 (same, to [1C], [1D], [1E]); EX2020 (Cooper Reply Decl. Tr.), 59:1-64:2, 77:11-84:17 (admitting reliance on Fig.11 for [1C], [1D], [1E]); see also EX2020 (Cooper Reply Decl. Tr.), 103:20-106:5 (mapping other fragmentation disclosures), 113:17-114:13; Slide 9.

Petitioner’s Sequencing Assertions Fail—Linnarsson Does Not Sequence the Entire Tagged Polynucleotide (*cont’d*)

- But *Linnarsson does not teach sequencing the entire polynucleotide. (cont’d)*
 - Petitioner’s three citations to Linnarsson provide no details and never identify *what* is sequenced, let alone show the *entire* tagged polynucleotide is sequenced.
 - Linnarsson’s Figure 1 is only an “*overview of a method*” that says “sequencing is performed” *without specifying what is sequenced*.
EX1003 (Linnarsson), 4:8-13; EX2013 (Quackenbush Decl.), ¶58; POR.24; SurRep.8.
 - Linnarsson’s 19:28-32 similarly indicates *something* is sequenced, *but not what* is sequenced.
POR.24; EX1003 (Linnarsson), 19:28-32; EX2013 (Quackenbush Decl.), ¶58.
 - Linnarsson’s 21:13-25 confirms the entirety of the tagged polynucleotide is not sequenced (only a *fragment* is) and discloses *further fragmenting* may be needed: “the cDNA library can be processed by fragmenting the cDNA library (e.g., with DNase) to obtain a short-fragment 5’-end library.”
POR.24-25; EX1003 (Linnarsson), 21:13-25; EX2013 (Quackenbush Decl.), ¶58; SurRep.11-12; see also Slide 18.
 - “cDNA library” is itself defined in Linnarsson as “a collection of cloned complementary DNA (cDNA) *fragments*.”
EX1003 (Linnarsson), 12:4-8; SurRep.11-12; EX2013 (Quackenbush Decl.), ¶57.

Linnarsson Teaches Sequencing the 5' Fragment

- Linnarsson's STRT method involves preparing a cDNA library for sequencing by reverse-transcribing mRNA initially produced by transcription of genes expressed in the cells in the sample.

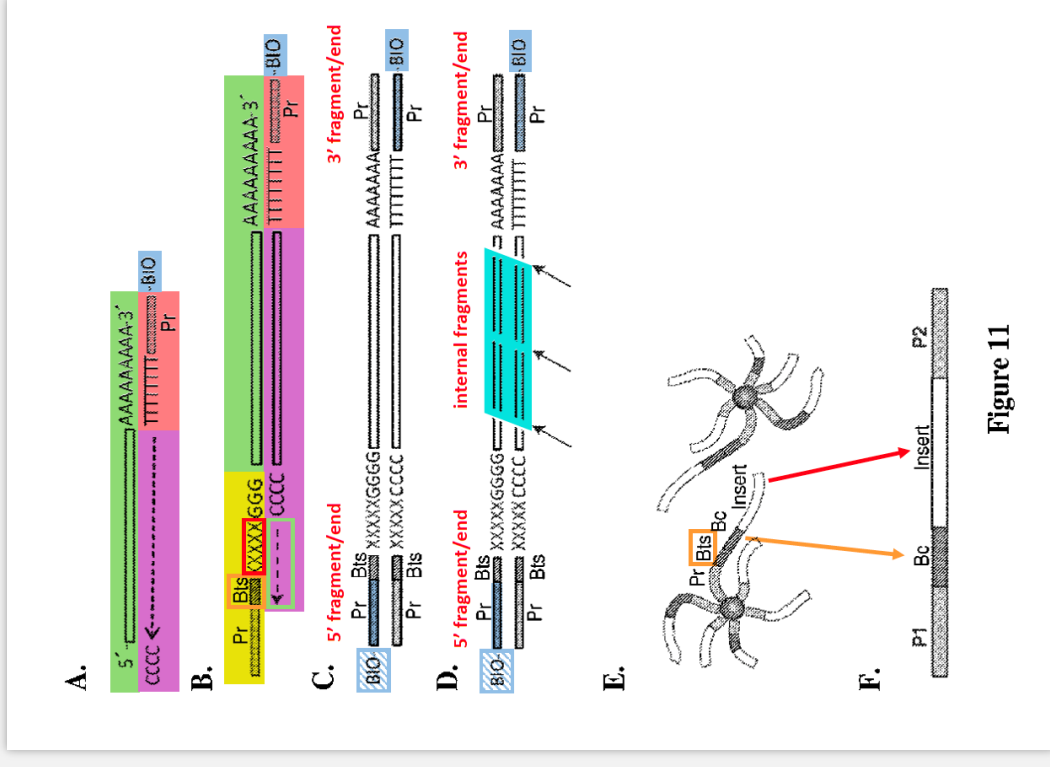
EX1003 (Linnarsson), 1 (Abstract), 3:25-4:2, 12:4-8, 5:10-11, 23:1-4, 37:31-32, Fig. 11; see also Pet.24; EX2013 (Quackenbush Decl.), ¶133-44; POR.7-9; SurRep.10-12.

- "single cells are placed in separate wells of a 96-well plate and lysed...."
- CDSs and TSOs are then incorporated into cDNA during reverse transcription from mRNA.
- After reverse transcription is completed, the cDNA (now including a CDS and TSO) from each well is amplified, at which a biotin is added to the 5' fragment, and then the cDNA is fragmented.

POR.11-12; SurRep.9-17; EX2013 (Quackenbush Decl.), ¶139; EX2018 (Cooper Dep. Tr.), 28:19-29:7.

- The biotinylated fragments, the "5' fragments" and "3' fragments," are bound to the beads and **the internal fragments are washed away.**
- The "5' fragment" is then released by BtsCI digestion, adapter sequences are added, and **it is sequenced, and the 3' fragment remains bound to the bead.**

EX1003 (Linnarsson), 6:5-12, 18:27-19:11; Pet.28; POR.12-13, 56-57; SurRep.9-17; EX2013 (Quackenbush Decl.), ¶141; EX2018 (Cooper Dep. Tr.), 31:12-22, 32:7-14, 43:21-44:22.



Petitioner's New Sequencing Reply Theories Fail

- Petitioner belatedly argued the sequencing limitation is met because “Linnarsson does not require fragmentation.”
Rep.5-6; EX1059 (Cooper Reply Decl.), ¶¶ 33-47, 64-73; SurRep.8-9.
- Dr. Cooper repeatedly argued Figures 3 and 4 confirm fragmentation is optional because “Figures 3 and 4 do not include... ***a restriction enzyme cut site at all....***”
E.g., EX1059 (Cooper Reply Decl.), ¶¶ 18, 42, 66-67 (testifying Figures 3 and 4 “do not include... any restriction enzyme cut site”); SurRep.9-10; see Slide 13.
- Dr. Cooper also testified that the “biotin (in the case of both the CDS and TSO) and restriction enzyme cut site (in the case of the TSO) are part and parcel of the fragmentation method used in Figure 11.”
EX1059 (Cooper Reply Decl.), ¶ 31; SurRep.9-10.
- But fragmentation is not optional in Linnarsson’s STRT method.
EX1003 (Linnarsson), 21:13-25, Fig. 11, 26:9-20, 35:17-27; EX2013 (Quackenbush Decl.), ¶ 58, 90-91; POR.24-25, 55-56 n.21; SurRep.9-14, 19; see Slides 6-17.
- Dr. Cooper ***admits he did no analysis to support his demonstrably false assertion that Figures 3 and 4 have no restriction enzyme cut site*** (and that a ***publication from reputable authors shows they do***).
See Slide 14; EX2101 (Guan), 1; EX2020 (Cooper Reply Dep. Tr.), 120:7-18, 133:22-137-10; SurRep.10.
- Consistent with Dr. Cooper’s “part and parcel” opinion, ***Figures 3 and 4 are part of the fragmentation and capture approach because they do have restriction enzyme cut sites.***
See Slides 6-17; EX2020 (Cooper Reply Dep. Tr.), 123:11-125:4, 129:2-7, 133:22-137:10; Rep.5; EX1059 (Cooper Reply Decl.), ¶ 31; SurRep.10-11.
- And this is consistent with Linnarsson, which ***introduces Figures 3 and 4 within Example 1, which uses fragmentation and capture.***
See Slides 6-17; EX1003 (Linnarsson), 23:1-24:20; EX2020 (Cooper Reply Dep. Tr.), 90:9-92:19, 94:8-97:11, 118:5-8 (admitting Ex.1 includes “fragmentation and capture”); SurRep.11.

Petitioner's New Sequencing Reply Theories Fail *(cont'd)*

- Petitioner and Dr. Cooper also belatedly assert Linnarsson Claims 1 and 27 are evidence that “Linnarsson does not require fragmentation.”
 - Petitioner argues “dependent claim 27 of Linnarsson, which recites ‘fragmenting,’” means fragmenting is not required.
Rep.6; EX1059 (Cooper Reply Decl.), ¶¶47, 72; EX2020 (Cooper Reply Dep. Tr.), 97:17-101:22, 111:8-113:16; SurRep.11-12.
 - Dr. Cooper also suggested for the first time in his post-Reply deposition that Linnarsson claim 1 evidenced fragmentation’s supposed optionality because it did not identify fragmentation as a requirement.
EX2020 (Cooper Reply Dep. Tr.), 130:22-132:2, 132:7-133:15; SurRep.11 n.6.
- But Linnarsson’s claims do not indicate fragmentation is not required.
 - Petitioner’s reliance on Linnarsson’s claim 27 is late, and its “fragmenting the cDNA library” language only confirms there may be an **additional** fragmentation step after creation of the cDNA library.
See Slide 18; Rep.6; EX2020 (Cooper Reply Dep. Tr.), 101:9-103:7; SurRep.11-12.
 - Linnarsson defines “cDNA library” as “a collection of cloned complementary DNA (cDNA) **fragments**.”
EX1003 (Linnarsson), 12:4-8; EX2020 (Cooper Reply Dep. Tr.), 97:17-98:20; POR.23; SurRep.12.
 - And Dr. Cooper concedes **additional** fragmentation may be required for, *e.g.*, the NGS or shotgun sequencing Linnarsson discloses.
See Slide 18; EX2020 (Cooper Reply Dep. Tr.), 111:8-113:16-103:7; EX1003 (Linnarsson), 22:5-21; SurRep.11-12.
 - Petitioner’s reliance on Linnarsson’s claim 1 is also late, and it does not exclude biotin or fragmentation: it is a “comprising claim” and is simply silent on those features.
EX2020 (Cooper Reply Dep. Tr.), 130:22-132:2, 132:7-133:15; EX1003 (Linnarsson), 46:3-12; SurRep.11 n.6.

Petitioner's New Sequencing Reply Theories Fail (cont'd)

- Further, whether or not fragmentation is required by Linnarsson's method *in general* (it is), **Petitioner and Cooper explicitly and repeatedly relied on Figure 11**, which indisputably incorporates the fragmentation and capture approach.

Pet.41-42, 45-47 (mapping Fig.11 to [1C], [1D]); EX1002 (Cooper Decl.), ¶¶139-141, 143, 153-156, 163-166 (same, to [1C], [1D], [1E]); EX2018 (Cooper Dep. Tr.), 23:15-25:21, 29:14-30:2; EX2020 (Cooper Reply Dep. Tr.), 59:1-64:2, 77:11-84:17; POR.55 n.21; SurRep.14-16; see also EX2020 (Cooper Reply Dep. Tr.), 103:20-106:5 (mapping other fragmentation disclosures), 113:17-114:13; EX2013 (Quackenbush Decl.), ¶¶36-44, 58; Slide 9.

- Dr. Cooper admitted he relied on Figure 11:

Q. And so, you rely on these steps [in Figure 11] and others described in Linnarsson in coming to your opinions about Linnarsson; is that correct?

A. **Correct. I use some of the -- you know, figure 11 is part of the evidence of -- of all of the disclosure that's in Linnarsson, yes.**

EX2018 (Cooper Dep. Tr.), 23:15-20, 29:14-30:2; POR.53, 55 n.21; SurRep.14-16; see also Slide 9.

- Indeed, both Petitioner and Dr. Cooper **repeatedly equated Figures 2 and 11, and treated them and Figures 3-4 as a single embodiment** without explaining how or why POSITA would mix-and-match across them.

Slide 9; Pet.26-29, 41-42 ("As shown in Figures 2 and 11... **Figure 11... discloses the same...**"), 42-43 ("As shown in Figure 2 below, **a TSO... Figure 11 discloses the same...** Figure 3... **similarly discloses that the TSO...**"), 45-47; EX1002 (Cooper Decl.), ¶¶139-141 ("As demonstrated in Figures 2 and 11, above, the CDS..."), 143 ("The TSO... is shown in Figures 2 and 11"), 153 ("Linnarsson's Figures 2 and 11, depicted below, **show...**"), 154-156 ("As can be seen below in Figures 2 and 11"), 163-166; EX2020 (Cooper Reply Dep. Tr.), 47:17-48:15, 76:13-78:19, 82:7-90:1; SurRep.14-15; EX2013 (Quackenbush Decl.), ¶189.

Petitioner’s New Sequencing Reply Theories Fail *(cont’d)*

- Petitioner’s Reply also belatedly argued that “‘sequencing’ a polynucleotide simply calls for generating sequence information from the nucleotide, not sequencing the ***entire*** molecule.”
Rep.4 (emphasis orig.); EX1059 (Cooper Reply Decl.), ¶¶ 7-8, 54-72; EX2020 (Cooper Reply Dep. Tr.), 15:7-17:15; SurRep.16-17.
- But even as to this improper new theory, Petitioner failed to show enough of the polynucleotide is sequenced to “generat[e] sequence information” as Petitioner argues.
Rep.4 (arguing term “simply calls for generating sequencing information”); POR.22-26; SurRep.16-17 (Reply arguments at “Rep.4-5” “[e]ven if considered... failed to address how much... would need to be sequenced, or attempt to show Linnarsson’s methods would sequence enough of it.”)..
- The Petition and Reply failed to address ***how much*** of the sample polynucleotide would need to be sequenced, or even attempt to show Linnarsson’s methods would sequence ***enough*** of it.
Pet.57; Rep.4; see EX1003 (Linnarsson), 4:8-13, 19:28-32, 21:13-25 (“This cDNA library can be sequenced to provide an analysis of gene expression in single cells or in a plurality of single cells.”); POR.22-26; SurRep.16-17.
- The Petition asserted only that “Linnarsson teaches that after generating the tagged polynucleotides, “sequencing is performed” thereon.
Pet.56-57; POR.22-26; SurRep.16-17.
- Dr. Cooper’s new testimony also does not explain how much of the tagged polynucleotide he argues is sequenced, or whether enough is sequenced in Linnarsson’s method to perform the limitations of the claim.
EX2020 (Cooper Reply Dep. Tr.), 15:7-17:15; Rep.4 (citing EX1059 (Cooper Reply Decl.), ¶¶ 7-8, 54-72); SurRep.16-17.

Petitioner's New Sequencing Reply Theories Fail *(cont'd)*

- All of these arguments also come *too late*.
 - Petitioner's new theories were not in its Petition, which asserted that sequencing is performed on the entirety of the tagged polynucleotide.
Pet.56-57; POR.22-26; EX2013 (Quackenbush Decl.), ¶¶54-60; Rep.4-5; SurRep.1, 16; Pap.25.
 - Petitioner's Reply asserts Linnarsson discloses sequencing the entire "tagged polynucleotide" based on its improper new argument that Linnarssons's fragmentation approach is optional.
Rep.5; EX1059 (Cooper Reply Decl.), ¶¶ 7-8, 54-72; SurRep.9-16; Pap.25.
 - Petitioner's Reply also improperly asserts for the first time that Linnarsson meets the sequencing limitation because it discloses sequencing a portion of the tagged polynucleotide.
Rep.3-4; SurRep.4-5, 16-17; EX1059 (Cooper Reply Decl.), ¶¶ 7-8, 54-72; EX2020 (Cooper Reply Dep. Tr.), 15:7-17:15; Pap.25.
 - These improper new theories, based on some previously unarticulated combination, should be rejected.
Pap.25; SurRep.2, 16-17.

Dr. Quackenbush's Sequencing Opinions Are Not "Inconsistent"

- Petitioner incorrectly claims Dr. Quackenbush has "taken directly contrary positions regarding the term 'sequencing the plurality of tagged polynucleotides.'" Pap.36
 - But it is Petitioner and Dr. Cooper who argued the "sequencing" limitation is met because Linnarsson teaches sequencing the entire polynucleotide. Slide 25; EX1002 (Cooper Decl.), ¶¶138, 146, 151, 197-199; Pet.56-57, EX2013 (Quackenbush Decl.), ¶¶54, 58, EX1057 (Quackenbush Dep. Tr.), 172:20-173:20; see also POR.22-26; SurRep.8-9, Pap.39, 1.
 - Dr. Quackenbush, in his testimony, interpreted Dr. Cooper's theory as requiring the entire polynucleotide to be sequenced: "**Dr. Cooper asserts** that *the 'tagged polynucleotides' in Linnarsson are the cDNA generated during reverse transcription, and that, in his opinion, these tagged polynucleotides are sequenced*." EX2013 (Quackenbush Decl.), ¶54 (citing EX1002 (Cooper Decl.), ¶¶138, 195-198); Pap.39, 1.
 - Dr. Quackenbush, **responding to Dr. Cooper's opinions**, explained that Dr. Cooper mischaracterizes Linnarsson, which does not teach sequencing of the entire polynucleotide, and that Dr. Cooper thus failed to established that Linnarsson teaches the "sequencing" limitation. EX2013 (Quackenbush Decl.), ¶¶54-58; see also POR.23, 25; Pap.39, 1.
 - In District Court, Dr. Quackenbush opined that **the language of the claims** does not require sequencing the entire polynucleotide. Pap.39, 1; EX1067 (Quackenbush District Court Report Excerpt), ¶¶160-161.

Ground 1: Petitioner's Combination Fails to Satisfy Its Argued Motivation to Combine Linnarsson and McCloskey and Reasonable Expectation of Success



Petitioner's Argued Motivation to Combine Depends on Tagging "Each" / "Every" / "Substantially All" mRNA Molecules

- Petitioner and Cooper asserted POSITA would have been motivated to modify Linnarsson to "solve" amplification bias (or "source-uncertainty") by tagging "each" / "every" / "substantially all" mRNA molecules in Linnarsson using McCloskey's teachings of a second tag.

Pet.53-55; EX1002 (Cooper Decl.), ¶192; EX2022 (Cooper IPR2023-00955 Decl.), ¶1222; Rep.12 n.5; EX2021 (IPR2023-00955 Petition), 72; see also POR.33; SurRep.5-6, Pet.10-11.
- "Linnarsson notes a problem with amplifying the cDNA... there is potential amplification bias."

Pet.54; EX1003 (Linnarsson), 2:3-13; EX1002 (Cooper Decl.), ¶190; POR.33; SurRep.5-6.
- "McCloskey... provides the exact solution. By incorporating a second tag in an oligonucleotide that *uniquely identifies each sample polynucleotide* prior to amplification, McCloskey *'solve[s] this source-uncertainty problem* for DNA sequences generated by standard PCR."

Pet.53-54; EX1002 (Cooper Decl.), ¶191; POR.33-34; SurRep.5-6.
- "To solve this [amplification bias] problem... *Every sample and the polynucleotides within every given sample* are first uniquely tagged before being amplified...."
- "Linnarsson describes a method *which is designed to be applied to substantially all mRNA molecules of a given cell*."

EX2022 (Cooper IPR2023-00955 Decl.), ¶1222 ('197's "sample polynucleotides" are thus "substantially all mRNA molecules of a given cell"); Rep.12 n.5; EX2021 (IPR2023-00955 Petition), 72; SurRep.5-6.

Petitioner's Theory Does Not Provide Enough Tags to Tag Each mRNA Molecule in Linnarsson

- Petitioner asserted in related proceedings that “It would have been a simple design choice to design the second tag sequences with appropriate number of nucleotides to ensure sufficient number of distinct second tag sequences in the reaction mixture (e.g., *at least 100 times [more] than the number of sample polynucleotides*) such that each sample polynucleotide is uniquely tagged.”
EX2008 (IPR2023-00958 Petition), ¶61 (citing EX2011 (IPR2023-00958 Cooper Declaration), ¶256); POR.35; SurRep.3-5.
- And according to Petitioner and its expert, there are between 50,000 and 300,000 mRNA molecules in any given mammalian cell.
EX2008 (IPR2023-00958 Petition), 61-62; EX2011 (IPR2023-00958 Cooper Declaration), ¶257; EX2012 (Marinov), 1; EX2013 (Quackenbush Decl.), ¶74; POR.2-3, 33-36; SurRep.2-5.
- Thus, tagging between 50,000 and 300,000 mRNA would require 5,000,000 to 30,000,000 tags.

POR.35, n.10; EX2008 (IPR2023-00958 Petition), 61-62; EX2011 (IPR2023-00958 Cooper Declaration), ¶257; EX2012 (Marinov), 1; EX2013 (Quackenbush Decl.), ¶74; SurRep.2-5.

Petitioner's Theory Does Not Provide Enough Tags to Tag Each mRNA Molecule in Linnarsson

- But Petitioner's theory (as confirmed by the Board's institution decision) relies on McCloskey's teaching of a **7 nucleotide long sequence** as the second tag...**which permits a maximum of 16,348 tags.**

EX1002 (Cooper Decl.), ¶192; Pet.52; ID.21-22; EX1004 (McCloskey), 4; SurRep.2-7; EX2013 (Quackenbush Decl.), ¶175; POR.33-36; SurRep.6-8.

"[T]he method would be identical to the approach already set for in Linnarsson, except that the tags in Linnarsson would be conceptually divided into two portions: one that codes for the unique cell and one that codes for the unique molecule."

Pet.55-56 (citing EX1002 (Cooper Decl.), ¶192).

"McCloskey discloses that its batch-stamp typically comprises 6 to 8 nucleotides, and its barcode comprises 7 nucleotides. Therefore, a POSA could have conceptually designated the first 6-8 nucleotides of Linnarsson's cell-tag sequence to identify the single-cell origin of the tagged polynucleotide, and use the next 7 nucleotides to distinguish the tagged polynucleotide from other one from the same single cell."

EX1002 (Cooper Decl.), ¶192.

- **Rather than dispute** that its proposed **7 nucleotide tag** would be **insufficient to tag the expected 50,000-300,000 molecules** in a mammalian cell, Petitioner makes **improper and failed new arguments regarding yeast and bacterial cells.**

POR.2-3, 34-36; Rep.11-14; see SurRep.2-9, 21.

Petitioner's Late Reliance on Yeast Cells Fails

- On Reply, Petitioner, through Dr. Cooper, first suggests McCloskey has enough tags to uniquely tag each mRNA molecule in a *yeast* cell.
Rep.11-12 (citing EX1059 (Cooper Reply Decl.), ¶187); SurRep.2-5.
- But Petitioner's new yeast theory fails because there are *still far too few tags in McCloskey*.
EX1004 (McCloskey), 4; SurRep.2-7.
- Per Cooper, "The total number of mRNA per cell in a yeast cell is between 15,000 and 60,000."
EX2008 (Petitioner's Petition in IPR2023-00958), 76 (100 times more); EX1059 (Cooper Reply Decl.), ¶187 (citing EX1063, 6); Rep.11-14; EX2011 (IPR2023-00958 Cooper Declaration), ¶1256; POR.33-36; SurRep.3.
- But Petitioner and Dr. Cooper do not dispute that they asserted POSITA would need *many times more tags* than the expected number of molecules to tag each molecule uniquely—Dr. Cooper opined POSITA would want *100 times more*.
EX2008 (Petitioner's Petition in IPR2023-00958), 76 (100 times more); Rep.11-14; EX2011 (IPR2023-00958 Cooper Declaration), ¶1256; POR.33-36; SurRep.3.
- By Petitioner's own arguments, would thus have needed *far more than the 16,348 tags McCloskey's seven-nucleotide barcode could provide* even for the low end of the expected mRNA in yeast cells (15,000).
EX1059 (Cooper Reply Decl.), ¶187; POR33-36; SurRep.2-7.

Petitioner's Late Reliance on Yeast Cells Fails (*cont'd*)

- The new yeast theory is also ***late and improper***.
 - Petitioner and Dr. Cooper never mentioned yeast cells in the Petition or opening declaration.
SurRep.2-3.
 - And Petitioner cannot fix flaws in its petition on reply by relying on new theories or evidence.

“Unlike district court litigation—where parties have greater freedom to revise and develop their arguments over time and in response to newly discovered material—the expedited nature of IPRs bring with it an obligation for petitioners to make their case in their petition to institute. While the Board's requirements are strict ones, they are requirements of which petitioners are aware when they seek to institute an IPR.”

Intelligent Bio-Sys., Inc. v. Illumina Cambridge, Ltd., 821 F.3d 1359, 1370 (Fed. Cir. 2016); *Wasica Fin. GmbH v. Cont'l Auto. Sys., Inc.*, 853 F.3d 1272, 1286 (Fed. Cir. 2017) (rejecting reply brief attempting to cure petition deficiencies, noting “obligation for petitioners to make their case in their petition”, noting that “Rather than explaining how its original petition was correct, Continental's subsequent arguments amount to an entirely new theory of *prima facie* obviousness absent from the petition.”) (citation omitted); *Apple Inc. v. Smart Mobile Techs., LLC*, IPR2022-00981, Pap.34, 35-36 (Nov. 7, 2023).

Petitioner's Late Reliance on Bacteria Cells Fails

- On Reply, Petitioner first argued McCloskey has enough tags to tag each mRNA molecule in a ***bacteria*** cell uniquely.
Rep.11-12; EX1059 (Cooper Reply Decl.), ¶¶86-87; EX2020 (Cooper Reply Dep. Tr.) 71:5-9, 76:13-3; SurRep.2-3
- But McCloskey's 7-nucleotide tag still does not provide enough tags to tag each mRNA molecule in a bacteria cell.
SurRep.4-5; see also POR.33-36; EX2013 (Quackenbush Decl.), ¶¶72-76.

"[B]acteria such as E. Coli have a little over 4,000 genes and ~1,380 mRNA molecules in each cell."

EX1059 (Cooper Reply Decl.), ¶87 (citing EX1061 (Moran), 1). SurRep.4-5.

- Thus, by Petitioner's own arguments, tagging each would require 138,000 tags.

SurRep.4-5; POR.33-36; EX2011 (IPR2023-00958 Cooper Declaration), ¶256; EX1001 ('981 Patent), 22:54-60.

Petitioner's Late Reliance on Bacteria Cells Fails (cont'd)

- In addition, Linnarsson's oligo-dT primer—which is what anneals to an mRNA molecule's poly-A tail—would **not** anneal to each mRNA molecule in a bacteria cell.
Pet.41-42 ([1C]), 44-47 ([1D]); EX2013 (Quackenbush Decl.), ¶¶34; EX2020 (Cooper Reply Dep. Tr.) 72:10-75:8; EX2102, (Wansgasnuwat), 1; SurRep.4.
- Petitioner's original mappings relied exclusively on annealing a CDS's thymine sequence **to the mRNA's poly-A tail (and only the poly-A tail)**.
Pet.41-42 ([1C]), 44-47 ([1D]); EX2020 (Cooper Reply Dep. Tr.) 71:5-9, 76:13-3; SurRep.2-5.
- But Cooper admits "in general, **the rule for bacteria is that they're not" polyadenylated**.
EX2020 (Cooper Reply Dep. Tr.), 72:10-75:8 (only "small percentage of the transcripts within any given bacteria" have poly-A tail); EX2102, (Wansgasnuwat), 1; SurRep.4.
- Thus, "each"/"every"/"substantially all" mRNA in bacteria could not be tagged by the CDSs identified in Petitioner's combination because the mRNA generally lack a poly-A tail.
Slide 35; SurRep.4-5.

- For any bacterial mRNA that might be polyadenylated, Cooper admits "I **don't think** [polyadenylation in prokaryotes is] used in quite the same way or has the... **same functionality**."

E.g., EX2020 (Cooper Reply Dep. Tr.), 74:18-76:3, 64:16-71:14, esp.64:16-65:1), 65:8-21(Cooper conceding he doesn't know, one way or the other, whether "mRNA polyadenylation is absent in prokaryotic mRNAs except in transcripts bound for degradation"), 66:5- 69:20 (same); EX2103 (Llorens-Rico), 4 ("polyadenylation is absent in prokaryotic mRNAs except in transcripts bound for degradation"); SurRep.4-5.

- Therefore, tagging these bacterial mRNA would not be analyzing gene expression—rather, it would at most identify, *e.g.*, transcripts bound for degradation.

SurRep.4-5; EX2020 (Cooper Reply Dep. Tr.), 64:16-65:1, 65:2-71:14, 74:18-76:3; EX2103 (Llorens-Rico), 4.

Many eukaryote-focusing scRNA-seq approaches rely on poly(T) primers to capture polyadenylated mRNAs. However, mRNA polyadenylation is absent in prokaryotic mRNAs, except in transcripts bound for degradation. One solution to this problem is

EX2103 (Llorens-Rico), 4.

Petitioner's Late Reliance on Bacteria Cells Fails (*cont'd*)

- The new bacteria theory is also *late and improper*.
 - Petitioner and Dr. Cooper never mentioned bacteria cells in the Petition or opening declaration.
 - And again, Petitioner cannot fix the flaws in its Petition on reply by relying on new theories or evidence.

EX2020 (Cooper Rep. Dep. Tr.), 71:5-9, 76:1-3; SurRep.2-3.

“Unlike district court litigation—where parties have greater freedom to revise and develop their arguments over time and in response to newly discovered material—the expedited nature of IPRs bring with it an obligation for petitioners to make their case in their petition to institute. While the Board's requirements are strict ones, they are requirements of which petitioners are aware when they seek to institute an IPR.”

Intelligent Bio-Sys., Inc. v. Illumina Cambridge, Ltd., 821 F.3d 1359, 1370 (Fed. Cir. 2016); *Wasica Fin. GmbH v. Cont'l Auto. Sys., Inc.*, 853 F.3d 1272, 1286 (Fed. Cir. 2017) (rejecting reply brief attempting to cure petition deficiencies, noting “obligation for petitioners to make their case in their petition”, noting that “Rather than explaining how its original petition was correct, Continental's subsequent arguments amount to an entirely new theory of prima facie obviousness absent from the petition.”) (citation omitted); *Apple Inc. v. Smart Mobile Techs., LLC*, IPR2022-00981, Pap.34, 35-36 (Nov. 7, 2023).

Tagging a Subset of Molecules Fails

- Petitioner argues the **claims** “do not require tagging *all* the mRNA molecules in such a cell.”
Rep.12; SurRep.5-6 & n.2.
- But Petitioner’s **motivation to combine does require tagging every mRNA molecule in the cell.**
SurRep.5; see also POR.33-36; see Slide 35.
- Petitioner never proposed any modification to Linnarsson to tag less than “substantially all mRNA molecules” in the cell, and never explained how amplification bias could be “solved” by tagging less than all.
POR.33-36; EX2013 (Quackenbush Decl.), ¶175; SurRep.6; Pet.53-55; EX2022 (Cooper IPR2023-00955 Decl.), ¶222.
- And **merely “reduc[ing]” amplification bias** (as Petitioner first suggests in Reply) is ***not sufficient for Petitioner’s theory, which depends on “solv[ing]” amplification bias.*** Petitioner expressly contrasted this against Linnarsson’s *reduction* of amplification bias.
Pet.54-55; POR.33 n.11; EX2013 (Quackenbush Decl.), ¶175; Rep.13-14; SurRep.5 n.2.

Ground 1: Petitioner's First Tag Theory Is Unclear



Petitioner's First Tag Theory Is Unclear

- Petitioner inconsistently maps the “first tag” to Linnarsson’s cell-tag and McCloskey’s batch-stamp.
- Petitioner first maps it to Linnarsson:

POR.26-32; SurRep.17-18.

“Thus, Linnarsson discloses that each tagged polynucleotide comprising a first tag sequence (i.e., the cell-tag) that correlates the identified polynucleotide with the single cell from which it is derived....”

Pet.47-49, 57 (mapping elements 1E and 1H to Linnarsson’s cell-tag); POR.26-32.

- Then Petitioner, relying on Dr. Cooper, imports McCloskey’s batch-stamp’s functionality as the “first tag sequence”:

“Indeed, as Dr. Cooper explains in the attached declaration, the method would be identical to the approach already set forth in Linnarsson, except that the tags in Linnarsson would be conceptually divided into two portions: one that codes for the unique cell and one that codes for the unique molecule.”

Pet.55-56 (citing EX1002 (Cooper Decl.), ¶192); EX2013 (Quackenbush Decl.), ¶61; POR.26-32; SurRep.17-18.

- Under Dr. Cooper’s “conceptually divided” theory “*the first portion performs the function of McCloskey’s ‘batch-stamp.’*”

EX1002 (Cooper Decl.), ¶192; EX2013 (Quackenbush Decl.), ¶61; POR.26-27.

- In addition to being inconsistent, McCloskey’s batch-stamp does not function as a “first tag sequence”—it is *identical* for all cells in a sample.

POR.28-32, 37-40; EX2018 (Cooper Dep. Tr.), 35:4-19; EX2013 (Quackenbush Decl.), ¶¶62-71.

Ground 2: Ligation Theory Fails To Establish a Motivation to Combine, and Adding Ligation to Petitioner’s Proposed Combination Fails to Meet the Limitations of Claim 5



Ground 2: Claim 5 Requires Ligation

- Claim 5 adds a ligation limitation:

5. The method of claim 1, **wherein the tagged polynucleotides are generated through at least one ligation reaction.**

EX1001, Cl.5.

The Petitioner's Ligation Theories Based on Ligating to the CDS Fail

- Petitioner asserts POSITA would have ligated a tag *to the end of the CDS*.
Pet.67-68; EX1002 (Cooper Decl.), ¶237; POR.53-57; SurRep.18-21.
- Ligation occurs when the DNA fragments' ends are joined by the formation of covalent bonds between the 3'-hydroxyl of one DNA terminus and the 5'-phosphoryl of another.
EX1001, 6:15-32; EX2013 (Quackenbush Decl.), ¶189; POR.53-55.
- But ligating to Linnarsson's CDS would fail because *the biotin on the CDS's 5' end would prevent ligation*.

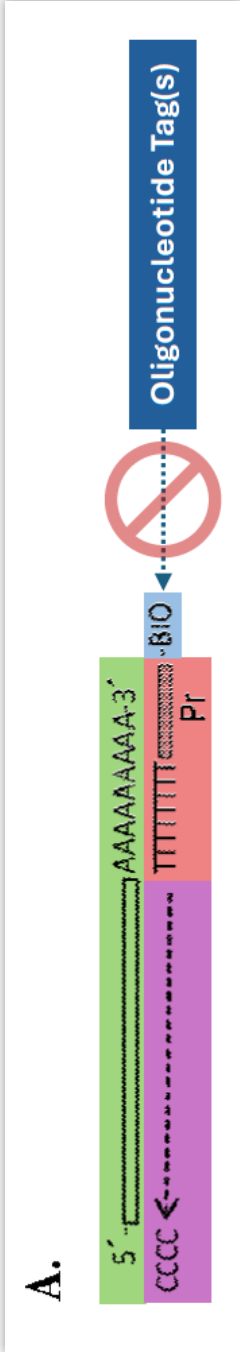
EX2013 (Quackenbush Decl.), ¶¶ 89-91; EX2018 (Cooper Dep. Tr.), 25:10-21; POR.53-55.

- Indeed, Petitioner *admits biotin*, if present, *would block ligation to the CDS*: "if one wanted to ligate a sequence to the CDS or TSO and still use biotin for fragmentation and capture, the biotin could not be at the end of the CDS or TSO"

Rep.22-24; EX1059 (Cooper Reply Decl.), ¶¶109-112; SurRep.18-21.

- And even if ligation *were possible, the CDS* in Figure 11, on which Petitioner and Dr. Cooper rely, does not include a Bts cut site and *remains bound to the bead* and would not be sequenced.

POR.55 n.21, 56-57; EX2013 (Quackenbush Decl.), ¶191; EX1003 (Linnarsson), 6:4-8, 27:6-8, Fig.11; EX2018 (Cooper Dep. Tr.), 43:21-44:22; Rep.24; SurRep.20-21.



The Petitioner's Ligation Theories Based on Ligating to the TSO Fail

- Petitioner argues POSITA would have ligated a tag *to the end of the TSO*.

Pet.67; EX1002 (Cooper Decl.), ¶237; POR.57-58; SurRep.18-21.

- Dr. Cooper's original declaration, relied on by the Petition, explained this ligation would happen "***after extension***."

EX1002 (Cooper Decl.), ¶243; Pet.68; EX2013 (Quackenbush Decl.), ¶¶94-95; POR.57-59.

- But ligating a tag to the TSO "**after extension**" means ligating after the ***first extension step*** (triggered by the CDS) and the ***second extension step*** (the template switching step triggered by the TSO) are completed.

EX2013 (Quackenbush Decl.), ¶¶94-95; EX2018 (Cooper Dep. Tr.), 21:21-32:14; POR.57.

- There is no third extension step that would then incorporate the ligated tag into the double stranded cDNA.
- In Reply, Petitioner and Dr. Cooper now make the improper new argument that "after extension" meant after just the first extension step. But this was not stated anywhere in the Petition or Dr. Cooper's original declaration: he admits he "did not explicitly say a timing element."

Rep.17; EX1059 (Cooper Reply Decl.), ¶115; POR.57-58; EX2018 (Cooper Dep. Tr.), 54:8-21; EX2013 (Quackenbush Decl.), ¶¶94-95; SurRep.23-24.

The Petitioner's Ligation Theories Based on Ligating to the TSO Fail

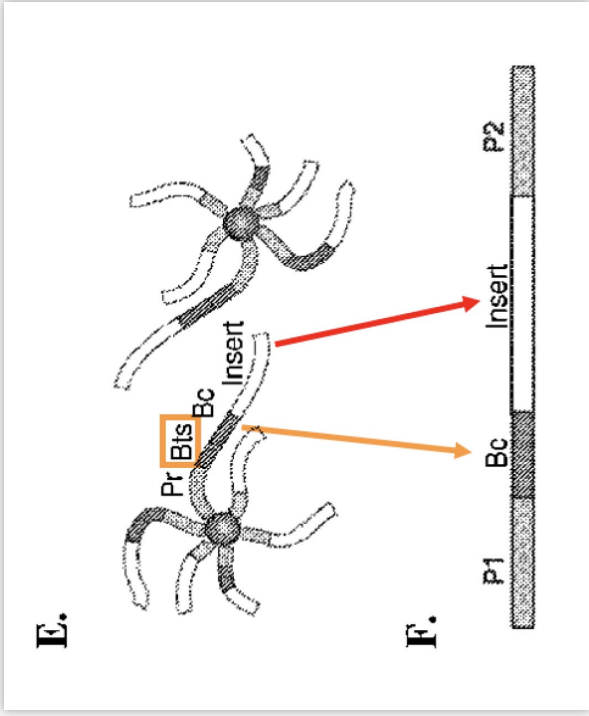
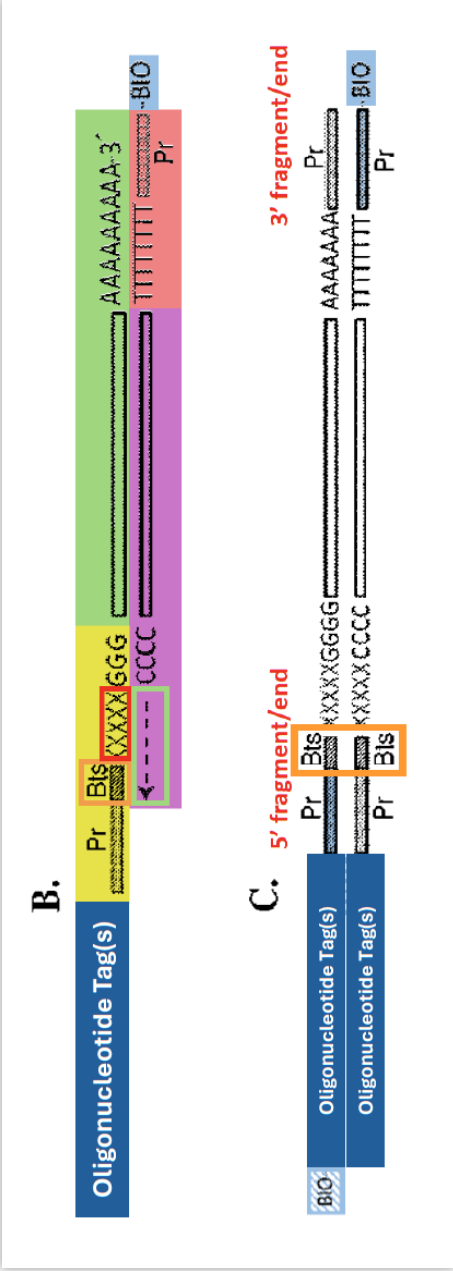
(cont'd)

- Even if considered, Petitioner does not dispute that *its argued ligation of the second tag to the TSO fails if Linnarsson's fragmentation and capture steps are included*, because **the second tag sequence would be on the 5' end of the restriction site and would remain bound to the bead and would not be sequenced.**

SurRep.20-21; POR.59; Rep.24; EX2013 (Quackenbush Decl.), ¶195; EX1059 (Cooper Reply Decl.), ¶118.

- Petitioner argues:

"a POSA would understand that if one wished to use fragmentation and capture through the use of a restriction enzyme, one would need to include the restriction enzyme cut site upstream (not downstream) of the molecular barcodes site so that the barcode is released from the capture bead for sequencing."



Rep.24

Petitioner's New Reply Arguments Regarding Optionality of Fragmentation and Capture With Biotin Fail

- On Reply, Petitioner argues for the first time that ligation would work because fragmentation and biotin are optional.

Rep.5-6, 23; EX2020 (Cooper Reply Dep. Tr.), 76:13-78:20 (admitting he “didn’t discuss the biotin molecule because I didn’t feel it was particularly important”), 82:7-84:17; EX1059 (Cooper Reply Decl.), ¶¶133-47, 64-73; SurRep.9-15, 19.
- But fragmentation and biotin are ***not*** optional in Linnarsson.

EX2013 (Quackenbush Decl.), ¶¶158, 90-91; POR.55-56 n.21; SurRep.9-14, 19; see Slides 6-17 .
- Among other things:
 - Linnarsson consistently discloses fragmentation and capture with biotin, including in Figure 11 and in both of its Examples.

See Slides 6-17; EX2013 (Quackenbush Decl.), ¶¶190-91; EX1003 (Linnarsson), Fig.11, 23:1-37:32; POR.55-56, 55 n.21; SurRep.9-14, 19.
 - Biotin is critical in Linnarsson’s method, as it allows for tagged polynucleotides to be isolated from untagged polynucleotides.

See Slide 7; POR.23-25; EX2013 (Quackenbush Decl.), ¶¶190-91; POR.55-56, 55 n.21; SurRep.9-14, 19.
 - Without such purification, tagged and untagged polynucleotides would be sequenced and the resulting information would be muddled.

SurRep.16; POR.12, 55-56, 55 n.21; EX2018 (Cooper Dep. Tr.), 31:12-22 (Figure 11E shows “a step that... they’re using to sort of clean up the products that they would like to sequence and separate them out from other things. So they’re... taking advantage of their cut sites and the beads that are connected to the biotin labels to help them clean up...”), 25:10-21 (biotin is “useful for a downstream step – to essentially separate out your molecules and clean them up”); EX2013 (Quackenbush Decl.), ¶190.

- As Dr. Quackenbush explains, “***Linnarsson discloses that the biotin captures the tagged fragments and enables their purification. ... Without biotin, all the fragments would be washed away.***”

EX2013 (Quackenbush Decl.), ¶190; SurRep.16; Rep.55 n.21.

Petitioner's New Reply Arguments Regarding the Optionality of Fragmentation and Biotin Fail (*cont'd*)

- Petitioner's arguments about Linnarsson's claims and Figures 3 and 4 fail to show fragmentation and biotin are optional in Linnarsson.

See Slides 9-16; Rep.6; EX1003 (Linnarsson), Figs.3-4; SurRep.10-11.

- And Petitioner and Dr. Cooper rely on Figure 11 and repeatedly *equated* Figures 2-4 and Figure 11.

See Slides 9, 30; EX2020 (Cooper Reply Dep. Tr.), 47:17-48:15, 76:13-78:19, 84:11-90:1; POR.55-56 & n.21; SurRep.14-16 & n.8.

- Petitioner and Dr. Cooper treated these disclosures as a single embodiment without explaining how or why POSITA would mix-and-match across them.

See Slides 9, 30; EX2020 (Cooper Reply Dep. Tr.), 47:17-48:15, 76:13-78:19, 84:11-90:1; SurRep.14-16 & n.8.

- Petitioner and Dr. Cooper never offered modifications to Linnarsson to eliminate its biotin or fragmentation, and never explained whether or how the method would work without these features

See Slide 19; POR.55-56 & n.21; SurRep.19; EX2013 (Quackenbush Decl.), ¶90.

Petitioner's Assertion That Patent Owner Is Arguing Bodily Incorporation Is Incorrect

- Petitioner asserts that the “test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference.”

Rep.24.

- But Patent Owner does not argue “bodily incorporat[ion].”

SurRep.19-20; POR.52-59; EX2013 (Quackenbush Decl.), ¶¶81-106.

- Rather, Patent Owner explained why *Petitioner’s proposed combination, “ligating to the CDS and TSO,” would not work and would not result in the claims being practiced* without further unspecified modifications to Linnarsson.

SurRep.19-20; POR.52-59; EX2013 (Quackenbush Decl.), ¶¶81-106.

- And, as the Board found in *Juniper*, explaining why Petitioner’s “proposed combination” would not work is *not* “engaging in an improper bodily incorporation.”

Juniper Networks, Inc. v. Swarm Tech. LLC, IPR2021-01445, Pap.62, 26-28 (Feb. 7, 2023); SurRep.19-20.

Petitioner's New Reply Arguments Further Modifying Ground 2 to Address Biotin and the Restriction Enzyme Cut Site Location Fail

- Petitioner newly argues in Reply that POSITA could have fixed the biotin issue by further modifying Ground 2 by **removing** the biotin from the CDS (or TSO) and **then “add[ing it] after ligation or includ[ing it] with the barcode.”**
See EX1059 (Cooper Reply Decl.), ¶¶ 109-112; see also EX1057 (Quackenbush Dep. Tr.) at 174:12-175:6; Rep.24; SurRep.20-21.
- Petitioner argues POSITA could have addressed the fact that ligated tags would not be released from the bead, and thus would not be sequenced, by further modifying Ground 2 by **“includ[ing] the restriction enzyme cut site upstream (not downstream) of the molecular barcodes site so that the barcode is released from the capture bead for sequencing.”**
See EX1059 (Cooper Reply Decl.), ¶¶ 113-118; Rep.24; SurRep.20-21.
- But, again, these are new modifications to Petitioner's combination and therefore comes too late.
SurRep.20-21; Juniper, IPR2021-01445, Pap.62, 26-28 (rejecting improper new reply evidence); Medtronic, Inc. v. Teleflex Innovations S.A.R.L., IPR2020-00127, Pap.107, 51-52 (June 17, 2021) (discounting reply arguments), *aff'd*, 70 F.4th 1331 (Fed. Cir. 2023); Dynaenergetics Eur. GmbH v. Swm Int'l LLC, IPR2022-01372, Pap.27, 49-50 (Feb. 5, 2024) (rejecting “meaningfully distinct” reply argument regarding proposed combination).
- These late-proposed modifications also fail because Petitioner again fails even to argue POSITA would have been motivated to modify Linnarsson in this manner or had a reasonable expectation of success.
SurRep.20-21; Rep.24.
- This is particularly so where Petitioner asserts these added complexities would merely achieve an “equivalent” method.
SurRep.20-21; POR.46-50; EX2013 (Quackenbush Decl.), ¶¶85-87; see Slide 57.
- Petitioner's extensive new modifications proposed in Reply confirm the non-obviousness of the claims. As the Board has previously observed:

“The extensive need to modify Kontos's device in a way not suggested in the Petition supports [PO's] argument that the proposed modifications are based on a hindsight desire to recreate the claimed invention, as opposed to a known need in the art for such a device...”

Medtronic, Inc. v. Teleflex Innovations S.A.R.L., IPR2020-00127, Pap.107, 51-52 (June 17, 2021), *aff'd* 70 F.4th 1331 (Fed. Cir. 2023); SurRep.20-21.

Hug's Linker Approach Does Not Cure the Issues of Ligating to the CDS or TSO

- The Petition also asserts that “[a] POSA could use Hug’s method as one exemplary method to ligate the oligonucleotide... a POSA could have designed a linker to hybridize to Linnarsson’s CDS and/or TSO as well as the oligonucleotide...”
Pet.68; POR.62-65.

- But Hug’s linkers are *not designed to link a series of random nucleotides*—like McCloskey’s barcode—*with another sequence*—like Linnarsson’s CDS or TSO.

POR.64-65; EX2013 (Quackenbush Decl.), ¶¶99-104; EX2018 (Cooper Dep. Tr.), 49:8-50:9.

- And Petitioner never explains how Hug’s linker approach could be modified to ligate a random sequence to the TSO or CDS.

POR.65; EX2013 (Quackenbush Decl.), ¶¶99-104.

- Regardless, Hug’s linker approach *does not permit a tag to be ligated to a biotin*.

POR.65; EX2013 (Quackenbush Decl.), ¶105.

- And Hug’s linker approach does not prevent the second tag from remaining bound to the bead if ligated to the TSO because it would still be ligated to the 5’ end of the restriction cut site, and thus not sequenced.

POR.65-68; EX2013 (Quackenbush Decl.), ¶¶91, 105; see Slide 50.

Petitioner's Improper New Ligating-to-the-Sample-Sequence Argument Also Fails

- Petitioner and Dr. Cooper cast their original Petition argument as asserting the second tag sequence is ligated “to the CDS or TSO.”
Pet.67-68; Rep.16 (describing Ground 2 theory as adding a second tag sequence); POR.46-47; SurRep.23.
- But on Reply, instead of ligating the “second tag” to the CDS or TSO, Cooper proposed ligating the second tag to the sample sequence after extension using Hug’s linker approach.
EX1059 (Cooper Reply Decl.), ¶¶113, 115; Rep.17 (citing EX1059, (Cooper Reply Decl.), ¶113); SurRep.23.

- Even if considered, Petitioner’s improper new theory fails to explain how those modifications would work, or what other modifications would be needed to permit the TSO to bind to the linker molecule, which Petitioner and Dr. Cooper do not explain.

SurRep.23.

Petitioner’s Newly-Argued Modifications Would Add Complexity Without Benefit

- Petitioner asserted “POSA would have been motivated to utilize this design choice because it would allow for added flexibility in selecting tag sequences during an experiment.”
Pet.68.
- But Petitioner, in its Reply, does not dispute litigation requires adding additional and complexity.
POR.46-47, 51-52; SurRep.21-22.
- And Petitioner argued the result would be “entirely equivalent,” which undermines any motivation to combine: “As Dr. Cooper explains, this approach would be *entirely equivalent to the method already disclosed by the combination of Linnarsson and McCloskey*, except that instead of having the tag sequence be part of the CDS or TSO prior to extension, the tag sequences are added to the CDS or TSO after extension.”
EX1002 (Cooper Decl.), ¶ 243.” Pet.68; POR.49-51; EX2013 (Quackenbush Decl.) ¶¶85-87; SurRep.20-22.
- POSITA would not be motivated to introduce complexity to achieve an equivalent method.
POR.46-52; SurRep.20.
 - *In re Schweickert*, 676 F. App’x 988, 995 (Fed. Cir. 2017) (non-precedential) (reversing Board’s obviousness determination where modification to prior art system that “already” performs function of proposed modification was “unnecessary” and “would do little more than add unwanted cost and complexity to the system”); *Weber, Inc. v. Provisur Techs., Inc.*, IPR2019-01461, Pap.36, 42-43 (Feb. 10, 2021) (finding no motivation to modify claim 1 combination to invalidate dependent claim where proposed modification doubled number of steps and added “needless complexity”) aff’d 2022 WL 17688071 (Fed. Cir. Dec. 15, 2022).
POR.51.
 - “It does not suffice to meet the motivation to combine requirement to recognize that two alternative arrangements...were both known in the art.” *Virtek Vision Int’l ULC v. Assembly Guidance Sys., Inc.*, 97 F.4th 882, 887-89 (Fed. Cir. 2024).
SurRep.21

Ground 2 Fails to Include Art Teaching Ligation, or Explain How and Why POSITA Would Modify Linnarsson

- Petitioner’s Ground 2 combination relies on knowledge of a POSITA to attempt to cover this ligation limitation.

Pet.65-66; POR.62-64.

- But Petitioner cannot rely merely on skill in the art to supply a missing limitation. And there must be an explanation as to **how and why POSITA would incorporate the teachings into the proposed combination**, and Petitioner failed to provide this.

POR.61-62, 65; SurRep.21-22.

“It does not suffice to meet the motivation to combine requirement to recognize the two alternative arrangements...were both known in the art.”

Virtek Vision Int’l ULC v. Assembly Guidance Sys., Inc., 97 F.4th 882, 886-87 (Fed. Cir. 2024); SurRep.21-22.

- Relying on the knowledge of a POSITA still requires a petitioner to establish POSITA would be **motivated to incorporate that knowledge** into the proposed combination, and to **explain how the teaching would be incorporated into the proposed combination** to render the claims obvious.

POR.62; *Applied Materials, Inc. v. Demaray LLC*, IPR2021-00103, Pap.52, 36-42 (June 29, 2022) (rejecting petitioner’s argument that references evidencing POSITA’s knowledge alone “sufficiently explain how such teachings apply to [the reference’s] system”); see also *DSS Tech. Mgmt., Inc. v. Apple Inc.*, 885 F.3d 1367, 1374 (Fed. Cir. 2018) (identifying “three caveats” to using common sense in obviousness analysis, warning it is not “typically invoked” “to supply a missing claim limitation”); *AI-Site Corp. v. VSI Int’l, Inc.*, 174 F.3d 1308, 1324 (Fed. Cir. 1999) (“Rarely, however, will the skill in the art component operate to supply missing knowledge or prior art to reach an obviousness judgment.”).

The Petition Theories Are Conclusory, Unclear, and Insufficiently Explained

- Petitioner’s assertion that ligation is a conventional technique does not mean any and all uses of ligation are automatically trivial or would work with in any and all approaches, nor does it absolve Petitioner of its burden of explaining a motivation to combine and REOS.
Pet.67 -68; SurRep.18-22; *Virtek*, 97 F.4th at 886-88 (“It does not suffice to meet the motivation to combine requirement to recognize that two alternative arrangements...were both known in the art.”); POR.63-65.
- Petitioner had to explain how the knowledge of skill, even as evidenced by a reference like Hug, would be applied to modify the combination.

POR.44 n.15; 62-65; SurRep.23-24.

The Petition Theories Are Conclusory, Unclear, and Insufficiently Explained (*cont'd*)

- The Petition’s Ground 2 theory builds from Ground 1’s obviousness combination. Pet.66; POR.41-42.
- The Petition asserted that, “[a]s Dr. Cooper explains, this approach would be entirely equivalent to the method already disclosed by the combination of Linnarsson and McCloskey, except that instead of having the tag sequence be part of the CDS or TSO prior to extension, the tag sequences are added to the CDS or TSO after extension.” Pet.68 (citing EX1002 (Cooper Decl.) ¶243; POR.49-52; SurRep.20-21.
- But Petitioner does not explain **what tag(s) would be added, to what** they would be added, **how** they would be added, **or when** they would be added. POR.41-44, 65-67.
 - Effectively acknowledging this, Dr. Cooper testified his report had ***implied*** that the second tag sequence added in Ground 1 was removed for Ground 2. EX2018 (Cooper Dep. Tr.), 58:22-59:10 (“**that was an obvious inference**”); POR, 42, 43 n.14.
 - And with respect to timing, Dr. Cooper admitted he ***did not specify*** when it occurred. EX2018 (Cooper Dep. Tr.), 54:8-21 (“**I did not explicitly say a timing element there.**”); EX2013 (Quackenbush Decl.), ¶106; POR.65-66; SurRep.25.
- This lack of particularly has deprived Patent Owner of due process, forcing Patent Owner to address multiple possible arguments never articulated by Petitioner and depriving Patent Owner of the ability to respond to whatever precise combination Petitioner might later claim it argued. POR.43 n.15; Harmonic Inc. v. Avid Tech., Inc., 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3)).

The Petition Theories Are Conclusory, Unclear, and Insufficiently Explained (*cont'd*)

- Petitioner’s arguments also fail to make clear how the ligation and subsequent steps (such as amplification) are implemented in Petitioner’s combination based on “knowledge of a POSA.”

POR.61-62; *Samsung Elecs. Co. v. Elm 3DS Innovations, LLC*, 925 F.3d 1373, 1380-81 (Fed. Cir. 2019) (affirming no REOS in part where petitioner failed to adequately explain how fabrication process would be changed to incorporate modification).

- When a “proposed combination is not at all clear” because it fails to provide a “‘reasoned analysis’ explaining what the combination is” the combination is neither clear nor workable, and cannot establish POSITA would have made the combination.

POR.43 n.15; *Samsung Elecs. Co. v. Lynk Labs, Inc.*, IPR2022-00101, Pap.30, 28-31 (May 25, 2023).

- And “cursory and unsupported” arguments that fail to explain “how and why [POSITA] would have modified [its references] with a teaching from [another reference]” cannot meet a petitioner’s burden.

POR.43 n.15; *ExtractionTek Sales LLC v. Gene Pool Techs., Inc.*, IPR2022-00832, Pap.32, 27-29 (Oct. 23, 2023).

Petitioner's Assertions About Increasing Flexibility Are Conclutory

- Petitioner asserted only that ligation “would allow for added flexibility in selecting tag sequences during an experiment,” and Dr. Cooper’s opening declaration merely parroted this conclusory assertion verbatim, with no explanation.

Pet.68; EX1002 (Cooper Decl.), ¶243; POR.50-52; SurRep.21-23.

- This unexplained, conclusory testimony cannot be credited.

POR.50-51; *Markforged Inc. v. Continuous Composites, Inc.*, IPR2022-00679, Pap.24, 28-29 (Sept. 26, 2023) (rejecting motivation to combine where “Petitioner’s statement [wa]s nothing more than an unsupported conclusory assertion regarding a possible benefit” without “providing the requisite rationale sufficient to support a conclusion of obviousness”); *Extraction Tek Sales LLC v. Gene Pool Techs., Inc.*, IPR2022-00832, Pap.32, 28-30 (Oct. 23, 2023) (concluding claims not unpatentable where petitioner and expert provided conclusory assertions of combination’s “advantage”); *Xerox Corp. v. Bytemark, Inc.*, IPR2022-00624, Pap.9, 15-16 (Aug. 24, 2022) (precedential) (“the cited declaration testimony is conclusory and unsupported, adds little to the conclusory assertion for which it is offered to support, and is entitled to little weight”) see also SurRep.22.

- As Dr. Quackenbush confirmed:

“Dr. Cooper has not identified any ‘flexibility’ that is gained when ligating a tag rather than, e.g., adding it via primer extension, which Dr. Cooper asserts would have been obvious before further modifying its combination with ligation. Assuming one could add a tag via ligation in Linnarsson’s method [], those same tag sequences could be added via primer extension with the CDS and/or TSO.”

EX2013 (Quackenbush Decl.), ¶86; POR.51-52; SurRep.22.

Petitioner's Assertions About Increasing Flexibility Are Conclutory

(cont'd)

- Dr. Cooper's reply declaration made the new assertion that adding tags via ligation provides flexibility to add shorter barcodes if tagging a small number of molecules.
EX1059 (Cooper Reply Decl.), ¶102; Rep.20-21.
- But even in his reply declaration Dr. Cooper *never explains how POSITA would know at the outset whether only a small number of endogenous molecules are present*, such that POSITA would know it could use shorter barcodes via ligation.
EX1059 (Cooper Reply Decl.), ¶102; SurRep.22.
- Indeed, *the very purpose of the claimed method is to determine that number of molecules in the sample—* POSITA would not know the number of molecules prior to tagging and sequencing.
SurRep.22.
- Further, neither Petitioner nor Dr. Cooper explain how this supposed added flexibility is specifically *achieved by ligation* as opposed to its Ground 1 combination.
EX1059 (Cooper Reply Decl.), ¶102; Rep.20-21; EX2013 (Quackenbush Decl.), ¶86; POR.51-52; SurRep.22.
- And, Petitioner argued the result would be “entirely equivalent,” which undermines any claim of additional flexibility.
Pet.68; EX2013 (Quackenbush Decl.), ¶¶85-87; POR.49-52; SurRep.22.

Petitioner's Analogous Art Analysis Is Deficient



Petitioner Failed to Establish Linnarsson and McCloskey Are Analogous to the '981

- The Petition argued McCloskey and Linnarsson are analogous to each other but failed to address whether they are analogous to the challenged patent.
Pet.52-53; EX1002 (Cooper Decl.), ¶187; POR.68; SurRep.27-28; see *Sanofi-Aventis Deutschland GmbH v. Mylan Pharms. Inc.*, 66 F.4th 1373, 1380 (Fed. Cir. 2023) (petitioner cannot meet its burden when it “argue[s] that [a prior art reference] is analogous to another prior art reference and not the challenged patent.”); see also SurRep.27-28.
- But Dr. Cooper claimed in deposition that **he had already provided a detailed explanation of how the asserted art was analogous to the '981 Patent** in paragraphs 27-37, 99-104, 108-117, and 186-194 of his first declaration.
EX2020 (Cooper Reply Dep. Tr.), 19:16-28:25; SurRep.27.
 - The paragraphs Dr. Cooper identified, however, are separate overviews of the '981 Patent (¶¶27-37), McCloskey (¶¶99-104), Linnarsson (¶¶108-117), and Dr. Cooper’s opinions relating to combining Linnarsson and McCloskey, including that those two references are analogous **to each other** (¶¶186-194).
EX1059, (Cooper Reply Decl.), ¶¶ 27-37, 99-104, 108-117, 186-194; SurRep.27.
- Unlike Dr. Cooper, Petitioner did not dispute that—while it affirmatively argued analogousness—it failed to assert analogousness **to the '981 patent** in its Petition.
Rep.24-25; SurRep.27.
- Instead, Petitioner asserts it can do so without limitation on Reply (citing *Sanofi-Aventis Deutschland GmbH v. Mylan Pharms.*, 66 F.4th 1373, 1379 (Fed. Cir. 2023)).
Rep.24-25 (citing EX1059 (Cooper Reply Decl.) ¶¶ 125-27); SurRep.27.
- But PO’s Response did not invite a broadening reply.

Cf. *Corephotonics, Ltd. v. Apple Inc.*, 84 F.4th 990, 1004-1010 (Fed. Cir. 2023) (declining to find Board abused discretion in allowing argument responding to patentee’s affirmative assertions against analogousness, noting, *inter alia*, “petitions were ambiguous; they did not make clear whether [petitioner] was stating [references] are in the same field... as the Challenged Patents or, instead, merely... one another”; petitioner permitted to respond both by **bolstering** its field of endeavor argument and by adding “pertinent-to-the-problem argument); SurRep.27-28.

Petitioner Failed to Establish McCloskey Is Prior Art



Petitioner Failed to Establish McCloskey Is Prior Art

- Petitioner asserted McCloskey (EX1004) is prior art ***based on EX1028***.

Pet.29 (citing EX1028 (Hall-Ellis Declaration)); Rep.3; POR.18; SurRep.25-27.

- But ***EX1028 does not even mention McCloskey***, let alone establish it was ***publicly accessible before*** the priority date.

EX1028 (Hall-Ellis Declaration); POR. 18.

- And McCloskey itself provides ***no admissible evidence it was disseminated or otherwise made publicly available prior to the priority date***.

SurRep.25-27.

- The ***date in McCloskey itself is hearsay***.

POR.18; SurRep.25-27; *Standard Innovation Corp. v. Lelo, Inc.*, IPR2014-00148, Pap.41, 18-28 (Apr. 23, 2015) (concluding claims not unpatentable where **reference not qualified as a printed publication** because, *inter alia*, **dates within the exhibit were inadmissible hearsay**); *Medivis, Inc. v. Novarad Corp.*, IPR2023-00042, Pap.35, 27-30 (Mar. 6, 2024) (copyright date “is not sufficient [even] at... institution... to demonstrate public accessibility”).

- And even if the hearsay date were considered, it would be ***insufficient to establish McCloskey was publicly accessible***.

SurRep.26-27; *Medivis, Inc. v. Novarad Corp.*, IPR2023-00042, Pap.35, 27-30 (Mar. 6, 2024) (copyright date and unsubstantiated argument that reference appeared online insufficient to demonstrate public accessibility).

Petitioner's Reply Arguments Should Not Be Considered

- Months later, on Reply, Petitioner first sought to fix its evidentiary failings with a brand new (late) Hall-Ellis Declaration:

"Nonetheless, supplied here is an authenticating declaration from Sylvia Hall-Ellis, further confirming McCloskey's prior art status. See EX1065 ¶¶ 44-45, 50. This is allowed on reply. See, e.g., *Hulu v. Sound View*, IPR2018-01039, Paper 29 at 15 (P.T.A.B. Dec. 20, 2019) (precedential)."

Rep.3; SurRep.25-27.

- To begin, the Reply's citation without discussion to the improper new Hall-Ellis declaration is also **improper incorporation by reference**, and should not be considered.

SurRep.25-27; Rep.3.

- Petitioner's improper new evidence is **not responsive to the POR**. As in *Sirius XM*, PO here "simply pointed out the defect in the Petition"—the Petitioner's failure to establish the reference was prior art.

Sirius XM, IPR2018-00690, Pap.63, 13-15 (refusing to consider reply evidence that reference was prior art where "Patent Owner's Response simply pointed out the defect in the Petition"); Rep.3; POR.18; SurRep.25-26

- Hulu, LLC v. Sound View Innovations, LLC*, IPR2018-01039, Pap.29, 15 (Dec. 20, 2019)—cited by Petitioner—does not support considering the new Hall-Ellis Declaration because it is not responsive evidence, as *Hulu* requires.

Rep.3; SurRep.26.

Patent Owner's Motion to Exclude



Patent Owner’s Motion to Exclude

- Patent Owner timely objected to McCloskey’s publication date as hearsay and irrelevant upon institution.

Pap.11 (PO’s Objections to Evidence); Pap.35 (PO’s Motion to Exclude), 1.

- Petitioner failed to serve supplemental evidence within the time period required under 37 C.F.R. § 42.64(b)(2).

Pap.35 (PO’s Motion to Exclude), 7-9.

- Instead, on Reply and without authorization, Petitioner purportedly “supplied [] an authenticating declaration from Sylvia Hall-Ellis, further confirming McCloskey’s prior art status.[] See EX1065 ¶¶ 44-45, 50.”

Rep.3.

- Petitioner further asserted “This is allowed on reply. See, e.g., *Hulu v. Sound View*, IPR2018-01039, Paper 29 at 15 (P.T.A.B. Dec. 20, 2019) (precedential).”

Rep.3.

- Patent Owner timely moved to exclude McCloskey (EX1004) and the new Hall-Ellis Declaration (EX1065), and any reliance thereon.

Pap.35 (PO’s Motion to Exclude), 1.

McCloskey Should Be Excluded

- Petitioner’s opposition identifies *no hearsay exception* that would permit McCloskey’s purported publication date to be considered for the truth of the matter asserted.

Pap.37 (Petitioner’s Opposition), 8–12; Pap.40 (PO’s Reply), 1–3; Fed.R.Evid.802.

- Instead of *hearsay*, the opposition argues *authentication*, asserting that “McCloskey is properly considered because it bears all conventional markers of publication,” and cites two Fodor Patents as supposedly confirming a date of publication.

Pap.37 (Petitioner’s Opposition), 8, 11 (citing EX1029 and EX1053); Pap.40 (PO’s Reply), 1.

- But “conventional markers” for *authentication* are *not pertinent to whether a date in McCloskey is hearsay* on a motion to exclude.

Pap.40 (PO’s Reply), 2–3.

- As to Petitioner’s argument that the Fodor Patents establish McCloskey as prior art:

- This argument was not included in the Petition, and is thus late and improper.
- And the Fodor Patents issued long after McCloskey’s 2009 priority date and simply repeat McCloskey’s hearsay date.

Pap.40 (PO’s Reply), 2–3.

Pap.40 (PO’s Reply), 2–3; EX1029, 3; EX1053, 4.

- Because it has not been established as prior art, McCloskey is irrelevant to the obviousness analysis—only prior art can be used to render a claim obvious.

Pap.35 (PO’s Motion to Exclude), 4–6; Fed.R.Evid.402.

The Hall-Ellis Declaration Is Untimely Supplemental Evidence and Should Be Excluded

- The Hall-Ellis Declaration is belated, unauthorized, supplemental evidence filed well past the deadline under 37 C.F.R. §§ 42.123, 42.223 and should be excluded.

Pap.35 (PO's Motion to Exclude), 7-9.
- Petitioner asserts that the Hall-Ellis Declaration is responsive evidence that was proper on reply.

Pap.37 (Petitioner's Opposition), 3-5; Pap.40 (PO's Reply), 3-5.
- But Patent Owner only pointed out a defect in the Petition—its failure to establish McCloskey was prior art—and this did not invite new evidence.

Pap.40 (PO's Reply), 3-5; see Slide 68; POR. 18.

 - The Board in *Sirius XM* similarly refused to consider reply evidence purportedly showing a reference was prior art because “Patent Owner’s Response simply pointed out the defect in the Petition.”

Sirius XM Radio Inc. v. Fraunhofer-Gesellschaft Zur Forderung Der Angewandten Forschung E.V., IPR2018-00690, Pap.63, 10, 13-15 (July 23, 2020); Pap.37 (Petitioner's Opposition), 7; Pap.40 (PO's Reply), 3-4.
- Petitioner also now requests for the Board to waive the requirements of 37 C.F.R. § 42.64(b)(2).
 - But Petitioner’s request is both late and unauthorized.

Pap.37 (Petitioner's Opposition), 7.
Pap.40 (PO's Reply), 5.
 - Petitioner waited over **five months** after Patent Owner objected to McCloskey and never sought permission to serve it late or file it as supplemental information.

Pap.40 (PO's Reply), 5.

Petitioner's Motion to Exclude



The Petitioner's Motion to Exclude

- Petitioner's Motion should be denied for failure to meet Rule 42.64(c)'s basic requirements for any motion to exclude, as it does not identify the objections in the record.

Pap.38 (PO's Opposition), 2-3.

- As the Board stated in *HTC Corp. v. Advanced Audio Devices, LLC*, IPR2014-01156, Pap.36, 24-26 (Dec. 29, 2015), the failure to “identify where in the record [the movant’s] objections to evidence were made prior to their being the basis of the Motion to Exclude, in violation of Rule 42.64(c),” renders a motion to exclude “procedurally deficient, and [it] may be denied on this basis alone.”

Pap.38 (PO's Opposition), 3.

- In its Reply, Petitioner states “ ...Petitioner inadvertently did not cite where its objections were stated in the record.”

Pap.41 (Petitioner's Reply), 1, 2-3.

The Petitioner’s Motion to Exclude (cont’d)

- Petitioner seeks to exclude EX2101 as irrelevant and prejudicial.
Pap.34 (Petitioner’s Motion to Exclude), 5-6.
- Dr. Cooper admits that EX2101 identifies GCAGTG as a restriction enzyme cut site:
 - “The Type IIS restriction endonuclease BtsI recognizes and digests at **GCAGTG** (2/0)”
 - “The cleavage sequence is **GCAGTG** (2/0), which means that BtsI cuts 2nt” – or nucleotides – “after the recognition sequence on the top strand and right after the recognition sequence on the bottom strand.”

Q. Does [EX2101] refresh your recollection one way or the other about whether GCAGTG is an enzyme restriction site?

A. Again, I'd have to read through this paper. I don't -- it's from New England Biolabs, and that's what they're saying, but I -- you know, it's hard to -- these are new England Biolabs authors in a PLOS One paper; I'd have to do more homework on this. I don't...

Q. And you didn't do that before you testified that there were no restriction sites in Figures 3 and 4 right?

A. I did not look at this paper.

EX2020 (Cooper Reply Dep. Tr.), 135:22-136:5, 136:13-137:10; Pap.38 (PO's Opposition), 8; see also SurRep.10-11.



OPEN @ ACCESS
Freely available online

Alteration of Sequence Specificity of the Type IIS Restriction Endonuclease BtsI

Shengxi Guan, Aine Blanchard, Penghua Zhang, Zhenyu Zhu^{*}
New England Biolabs, Inc., Ipswich, Massachusetts, United States of America

Abstract
The Type IIS restriction endonuclease BtsI recognizes and digests at GCAGTG(2/0). It comprises two subunits: BtsA and BtsB. The BtsB subunit contains the recognition domain, one catalytic domain for bottom strand nicking and part of the cleavage domain. The BtsA subunit contains the cleavage domain and the other catalytic domain for top strand nicking. BtsA alone has no activity unless it mixes with BtsB to reconstitute the BtsI activity. During characterization of the enzyme, we identified a BtsB mutant R119A found to have a different digestion pattern from the wild type BtsI. After characterization, we found that BtsB(R119A) is a novel restriction enzyme with a previously unreported recognition sequence CAGTGG(2/0), which is named as BtsI-1. Compared with wild type BtsI, BtsI-1 showed different relative activities in NEB restriction enzyme reaction buffers NEB1, NEB2, NEB3 and NEB4 and less star activity. Similar to the wild type BtsB subunit, the BtsI-1 B subunit alone can act as a bottom nicking enzyme recognizing CAGTG(-/0). This is the first successful case of a specificity change among this restriction endonuclease type.

Citation: Guan S, Blanchard A, Zhang P, Zhu Z (2010) Alteration of Sequence Specificity of the Type IIS Restriction Endonuclease BtsI. PLoS ONE 5(7): e11787. doi:10.1371/journal.pone.0117187

Editor: Fernando Rodrigues-Lima, University Paris Diderot-Paris 7, France

Received: April 2, 2010 **Accepted:** June 30, 2010 **Published:** July 26, 2010

Copyright: © 2010 Guan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: New England Biolabs supported the work. As the authors are all employed by New England Biolabs, the funders were involved in study design, data collection and analysis, decision to publish and preparation of the manuscript.

Competing Interests: The engineered products, BtsI-1 and BtsI-2, which can be commercial products, are not for sale. This does not affect the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* Email: zhu@neb.com

Introduction

Restriction-Modification (RM) systems mostly occur naturally in bacteria and serve as defensive systems against invading DNA, particularly bacteriophages. The restriction endonuclease digests invading DNA while the DNA methyltransferase component(s) (cognate methyltransferase(s)) modifies the host genome and protects the host DNA from restriction endonuclease digestion [1]. There are groups of restriction endonucleases (Rases): Type I, Type II, Type III and Type IV [2]. Among them, the Type II Rases are the most widely used and best studied and used as tools in molecular biology, largely due to their well-defined recognition and cleavage sites. So far, more than 3800 Type II Rases with 299 distinct specificities have been characterized biochemically or genetically [3]. Among the subdivisions of Type II Rases, the Type IIS are unique in having asymmetric recognition sequences [2].

BtsI (Genbank: C808224.1), a Type IIS REase, was isolated from *Bacillus thuringiensis* (Pan, X.S., Morgan, R., unpublished observation). BtsI REase consists of two subunits BtsA and BtsB, and the modification system also contains two methyltransferases (M1.BtsI and M2.BtsI) [4]. Each methyltransferase modifies one strand of the recognition sequence. BtsB, the large subunit, contains a DNA recognition domain and a catalytic domain that can act naturally as a bottom strand nicking enzyme. The recognition sequence is written as GCAGTG(-/0), which means that BtsB does not cut the top strand and cuts right after the recognition sequence on the bottom strand. BtsA, the small subunit, contains part of the catalytic domain responsible for top strand cleavage. BtsA alone is inactive. Only when BtsA is

combined with BtsB do they form a restriction endonuclease capable of double-strand cleavage (GCAGTG(2/0)) [4]. The cleavage sequence is GCAGTG(2/0), which means that BtsI cuts 2 nt after the recognition sequence on the top strand and right after the recognition sequence on the bottom strand.

Numerous efforts have been made to engineer REases for altered specificity but with rather few successful examples. Several groups reported success in altering the specificity of Type IIG REases. For example, EcoO1 (CTGAGAG(6/14)) has been engineered to EcoS/M1 (CTGAGAG(6/14)) by random mutagenesis followed by challenges with EcoS71 (CTGAGAG(6/14)) and GsaI (CTGAGAG(6/14)) [5]. EcoO1 (CTGAGAG(6/14)) and GsaI (CTGAGAG(6/14)) were then used to engineer a Type IIG-like REase that can recognize and cleave DNA with a new recognition and digestion specificity by domain swapping with another similar Type IIG REase [6]. One major class of successful examples is from MneI(TCCRAC(20/18)) or TCCRAC(21/19)-like Type IIG REases. By analyzing the amino acid sequence of a group of closely related REases, specificity can be rationally altered by changing the corresponding amino acid residues in the protein [7]. All of these successful examples are Type IIG REases, in which endonuclease, methylase, and specificity domains are fused into a single polypeptide chain.

Other than Type IIG REases, there are reports of incomplete specificity changes with certain preference for one particular sequence. For example, some mutants of BstBII change specificity from C/TCGRGG to prefer C/CGGGG, C/TCGGG or C/TCGAG[8]. BstYI has been engineered to favor A/GAATCT than A/GATG [9]. HincII (Q38P) shows a stronger preference for GTT/AAC, in which GTG/GAC than wild type enzyme [10]. However, engineering the catalytic domain is relatively easier than engineering the recognition

The Petitioner's Motion to Exclude (cont'd)

- And Cooper also admits that GCAGTG is found in both Figures 3 and 4 of Linnarsson:

EX2020 (Cooper Reply Dep. Tr.), 121:2-21, 133:22-134:20; Pap.38 (PO's Opposition), 4, 7-8; see Slides 12-16.

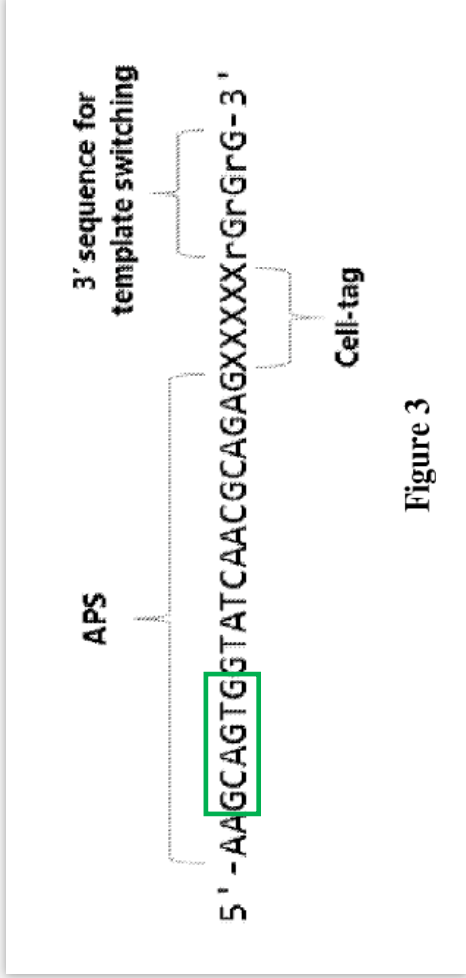


Figure 3

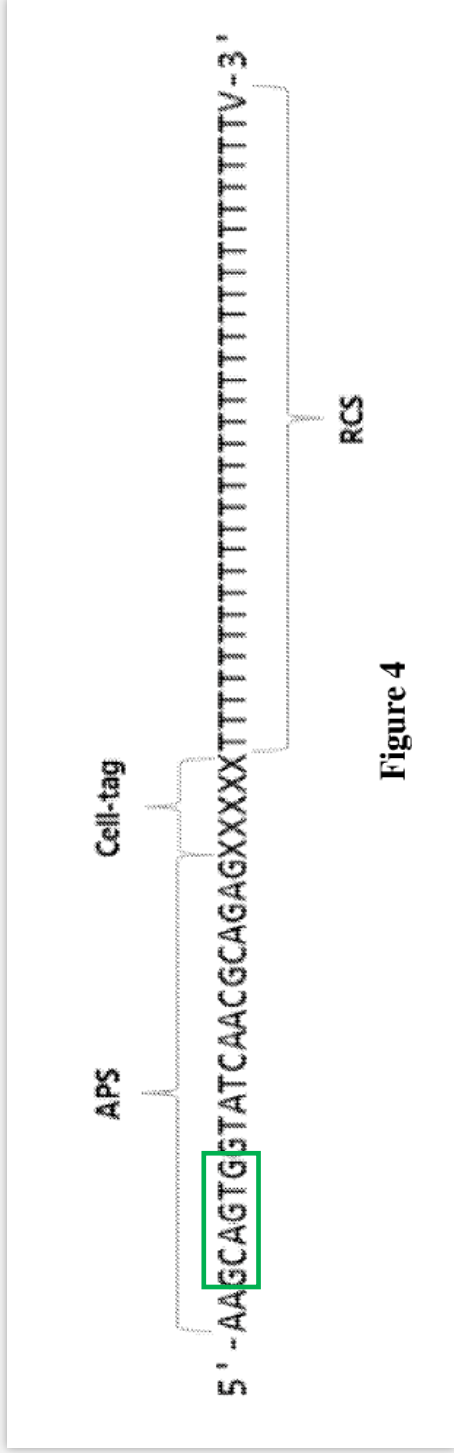


Figure 4

EX1003 (Linnarsson), Figs.3-4.

The Petitioner's Motion to Exclude (*cont'd*)

- EX2101 is **clearly relevant** because it **contradicts Petitioner's substantive arguments**.

"Figures 3 and 4 do not include a biotin or a restriction enzyme cut site at all, thus confirming that Linnarsson teaches approaches that do not require them."

EX1059 (Cooper Reply Decl.), ¶118; Pap.38 (PO's Opposition), 5-7; see Slides 12-16.

"Linnarsson, however, contains numerous other exemplary embodiments that ***do not*** include... any restriction enzyme cut site.... Linnarsson's Figure 3 and Figure 4 show exemplary TSO and CDS that... do not contain any restriction enzyme cut site."

EX1059 (Cooper Reply Decl.), ¶66 (bold italics original); Pap.38 (PO's Opposition), 6-7; see Slides 12-16.

"The lack of a restriction enzyme cut site... in Figure 3 is critical because it shows that Linnarsson is not limited to the fragment and capture approach of Figure 11."

EX1059 (Cooper Reply Decl.), ¶42; Pap.38 (PO's Opposition), 6-7; see Slides 12-16.

"As I explain above, where no biotin and no restriction enzyme cut site are present, the embodiment does not involve fragmenting the tagged polynucleotides as disclosed in Figure 11."

EX1059 Cooper Reply Decl.), ¶67; Pap.38 (PO's Opposition), 6-7; see Slides 12-16.

The Petitioner's Motion to Exclude (cont'd)

- Thus, *Petitioner and Dr. Cooper clearly put at issue whether there is any restriction enzyme cut site in Figures 3 and 4, and EX2101 (showing that there is) is clearly probative of whether Dr. Cooper's opinions are true or false.*

Pap.38 (PO's Opposition), 4, 7-12; Fed.R.Evid.401 ("Evidence is relevant if: (a) it has any tendency to make a fact more or less probable than it would be without the evidence; and (b) the fact is of consequence in determining the action.")

- EX2101 also gives context to Dr. Cooper's deposition testimony regarding whether Figures 3 and 4 have any restriction enzyme cut site and what Dr. Cooper actually did (or did not do) before testifying—incorrectly—in his declaration that they lack a cut site.

Pap.38 (PO's Opposition), 6-8; EX1003 (Linnarsson), Figs.3-4; EX2101 (Guan), 1; EX2020 (Cooper Reply Dep. Tr.), 120:7-121:16 (admitting he "did not" "look for any other cut site" other than "the cut site that is referred to in Figure 11"), 121:22-122:14, 123:11-124:21, 125:5-22, 127:21-129:7, 133:19-137:10; see Slides 12-16.

- Indeed, the significance of Dr. Cooper's testimony contradicted by EX2101 is clear: the only other embodiment Dr. Cooper identified to support his assertion that Linnarsson's fragmentation and capture approach was optional was Linnarsson claim 1, but the breadth of claim 1 is not a disclosure of an embodiment without fragmentation.

See, e.g., *In re Benno*, 768 F.2d 1340, 1345-46 (Fed. Cir. 1985 ("The scope of a patent's claims determined what infringes the patent; it is not measure of what it discloses."); Pap.38 (PO's Opposition), 6-9 & n.4.

The Petitioner's Motion to Exclude (cont'd)

- Petitioner also argues EX2101 should be excluded because of the danger of unfair prejudice or confusion that could be caused because Bts “happens to have a name highly similar to the enzyme that is used in Linnarsson,” BtsCl.
Pap.34 (Petitioner's Motion to Exclude), 6-7.
- But there is no danger of unfair prejudice or confusion:
Pap.34 (Petitioner's Motion to Exclude), 7.

– Unlike a lay jury, “the Board is well-positioned to determine and assign appropriate weight to the evidence.

See *Medtronic Vascular, Inc. v. TMT Sys., Inc.*, IPR2021-01532, Pap.56, 46-48 (Mar. 7, 2023); *Spinal Elements, Inc. v. Spectrum Spine IP Holdings, LLC*, PGR2021-00050, Pap.47, 54-55 (Aug. 17, 2022) (“Rule 403 has limited applicability, if any, to bench trials like this proceeding”); *Fox Factory, Inc. v. SRAM, LLC*, IPR2017-00472, Pap. 64, 60 (Apr. 18, 2018) (“As the factfinder, we are able to consider this evidence, in light of the parties’ arguments, and give it the appropriate weight.”); *Neste Oil Oyj v. Reg Synthetic Fuels, LLC*, IPR2013-00578, Pap.53, 10-11 (Mar. 12, 2015) (proceedings “before the Board are not jury trials; in the absence of a jury, the risk of unfair prejudice against which Rule 403 guards is diminished, if not eliminated entirely”); see also *United States v. Preston*, 706 F.3d 1106, 1117 (9th Cir. 2013), *rev'd on other grounds*, 751 F.3d 1008 (9th Cir. 2014) (*en banc*) (“Rule 403 is inapplicable to bench trials.”); Pap.38, 13.

– And Patent Owner's Sur-Reply is clear that the BtsI enzyme and the BtsCl enzyme are not the same.

Pap.38 (PO's Opposition), 13; SurRep.10-11.

- Petitioner also asserts that, in addition to EX2101, arguments in Patent Owner's Sur-Reply relying on EX2101 should be excluded. But Patent Owner's Sur-Reply Arguments regarding Figures 3 and 4 are supported by, *inter alia*, Dr. Cooper's deposition testimony *prior* to the introduction of EX2101, and should not be excluded for that additional reason.
Pap.38 (PO's Opposition), 3 n.2.

Petitioner's Reply Should Be Ignored Based on the Extent of Improper Out-of-Scope Reply Arguments



Petitioner's Reply Should Be Ignored Based on the Extent of Improper Out-of-Scope Reply Arguments

"Examples of indications that a new issue has been raised in a reply include new evidence necessary to make out a prima facie case for the patentability or unpatentability of an original or proposed substitute claim, such as newly raised rationale to combine the prior art references that was not expressed in the petition. ... It is also improper for a reply to present new evidence (including new expert testimony) that could have been presented in a prior filing, for example newly cited prior art references intended to 'gap-fill' ... with 'newly cited' art"

Consolidated Trial Practice Guide (PTAB, Nov. 2019), 74; SurRep.2.

"Once the Board identifies new issues presented for the first time in reply, the Board 'will not attempt to sort proper from improper portions of the reply'.... We do not consider the Reply."

Sirius XM Radio Inc. v. Fraunhofer-Gesellschaft Zur Forderung Der Angewandten Forschung E.V., IPR2018-00690, Pap.63, 13-15 (July 23, 2020); *SurRep.2*

"IBS supported its new theory of invalidity by reference to new evidence, citing 'a number of non-patent literature references which were not relied upon to support unpatentability in the Petition'.... In these circumstances, we find that the Board did not err in refusing the reply brief as improper under 37 C.F.R. § 42.23(b) because IBS relied on an entirely new rationale to explain why one of skill in the art would have been motivated to combine Tsien or Ju with a modification of Zavgorodny."

Intelligent Bio-Sys., Inc. v. Illumina Cambridge, Ltd., 821 F.3d 1359, 1369-70 (Fed. Cir. 2016) ; *SurRep.3*.

Petitioner’s New Argument that the Linnarsson-McCloskey Combination’s Second Tag (Barcode) Is Not Limited to “7 Nucleotides” Was Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Linnarsson-McCloskey combination used McCloskey’s second tag (barcode) of “7 nucleotides.” EX1002, ¶192 (“McCloskey[’s]... barcode comprises 7 nucleotides... POSA could...use... 7 nucleotides...”); Pet.55-56 (citing EX1002, ¶192).	Linnarsson-McCloskey combination’s second tag (barcode) <i>not limited to 7 nucleotides</i> ; 7 nucleotide length (16,384 barcodes) “just an example in McCloskey.” Rep.13; EX1059, ¶¶83-84; see, e.g., EX2020, 45:1-58:1, esp. 57:17-58:1.

Pap.25, 1.

- Petitioner first argued the second tag (barcode) in its proposed combination was McCloskey’s 7 nucleotide barcode.
- Pet.55-56 (citing EX1002 (Cooper Decl.), ¶192).

- But Petitioner now argues on Reply that the second tag (barcode) is not limited to “7 nucleotides” and Dr. Cooper testified that “McCloskey includes a general formula for how long the barcodes must be to distinguish a specific number of molecules,” 4ⁿ.

Rep.13 (including no cites to Dr. Cooper’s first declaration); EX1059 (Cooper Reply Decl.), ¶¶83-84; see, e.g., EX2020 (Cooper Reply Dep. Tr.), 45:1-58:1, esp. 57:17-58:1; see also SurRep.6-8, 25.

Petitioner’s New Argument to Modify McCloskey’s Barcode to “20-30” Nucleotides Based on New Reference and Declaration from Another IPR Was Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
No reliance on McCloskey application US2007002640 (“McCloskey II”) in Petition or Cooper’s original declaration. See generally Pet.; EX1002. See, e.g., EX2020, 58:2-22.	Reply and Cooper’s reply declaration relying on McCloskey II and testimony from a different IPR to modify McCloskey’s barcode to “20-30” nucleotides. Rep.13-14 (relying on additional opinions in EX2011); EX1059, ¶85.

Pap.25, 1.

- The Petition did not rely on the McCloskey application US2007002640 (“McCloskey II”) or on Cooper’s testimony from a different IPR.
- But Petitioner and Dr. Cooper now rely, in Reply, on McCloskey II and Dr. Cooper’s testimony from a different IPR to modify the barcode to “20-30” nucleotides.

See generally Pet.; EX1002 (Cooper Decl.); see, e.g., EX2020 (Cooper Reply Dep. Tr.), 58:2-22.

Rep.13-14 (relying on Cooper opinions from different IPR in EX2011); EX1059 (Cooper Reply Decl.), ¶85 (relying on McCloskey II).

Petitioner’s New Argument Disclaiming Reliance on CDS/TSO Fragmentation and Capture Examples, and First Arguing Separate Optional Embodiments, Was Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Mapping CDS/TSO fragmentation and capture examples, including biotin and restriction enzyme cut sites, to tagged polynucleotide limitations; Fig.11 mapped together with Figs.2-4 as single embodiment (no discussion /explanation of mapping across any separate embodiments); no argued disclosure based on Linnarsson claim 1 or arguments in-volving Linnarsson claim 27. Pet.41-43, 45-47; EX1002, ¶¶139-141, 143-144 ([1C]), 153-155 ([1D]), 163-166 ([1E]); see also EX1002, ¶198 (fragmentation in [1G]); see, e.g., EX2020, 76:13-90:1, 90:9-92:19, 94:8-97:11, 104:2-106:5.	Disclaiming reliance on CDS/TSO frag-mentation and capture examples, arguing biotin and restriction enzyme cut sites are optional and not used in Linnarsson Figs.2-4 (or, per deposition, Linnarsson claim 1); Figs.2-4 (and, per deposition, Linnarsson claim 1) are different embod-iments from Fig.11. Rep.5-7, 23-24; EX1059, ¶¶33-45, 66-70; see, e.g., EX2020, 118:5-122:14, 123:11-125:4, 129:2-7, 131:20-137:10. Also arguing Linnarsson claim 27 indicates fragmen-tation is optional in these embodiments. Rep.6; EX1059, ¶¶47, 72.

Pap.25, 1.

- Petitioner originally mapped the tagged polynucleotide limitations to CDS/TSO fragmentation and capture examples using Fig.11 together with Figs.2-4 as a single embodiment (without arguing or explaining how features relied on from each Figure would have been treated as or combined across the Figures as separate embodiments), and never argued disclosure based on Linnarsson claim 1 or optionality arguments involving Linnarsson claim 27.
- But Petitioner on Reply now disclaims reliance on CDS/TSO fragmentation and capture examples, arguing biotin and restriction enzyme cut sites are optional and not used in Linnarsson Figs.2-4 embodiments (or, per deposition, Linnarsson claim 1), and that Figure 11 is now a different embodiment. Petitioner also now argues claim 27 evidences optionality.

Pet.41-43, 45-47; EX1002 (Cooper Decl.), ¶¶139-141, 143-144 ([1C]), 153-155 ([1D]), 163-166 ([1E]); see also EX1002 (Cooper Decl.), ¶198 (fragmentation in [1G]); see, e.g., EX2020 (Cooper Reply Dep. Tr.), 76:13-90:1, 90:9-92:19, 94:8-97:11, 104:2-106:5; see Slides 9, 19, 29-30.

Rep.5-7, 23-24; EX1059 (Cooper Reply Decl.), ¶¶33-45, 66-70; see, e.g., EX2020 (Cooper Reply Dep. Tr.), 118:5-122:14, 123:11-125:4, 129:2-7, 131:20-137:10. Also arguing Linnarsson claim 27 indicates fragmentation is optional in these embodiments. Rep.6; EX1059 (Cooper Reply Decl.), ¶¶47, 72; see Slides 9-17, 28-30, 32.

Petitioner’s New Arguments For Modifying the CDS/TSO to Enable Ligation Were Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Tag ligated to the end of the CDS/TSO with no identified modification to CDS/TSO having biotin and enzyme restriction cut sites. Pet.68 (Ground 2); EX1002 ¶243.	Ligation accomplished by avoiding fragmentation and capture disclosures with biotin and enzyme restriction cut sites (see above) or by making new modifications to the CDS/TSO—e.g., removing biotin, adding it later, moving the Bts cut site, etc. Rep.24; EX1059, ¶¶109-112, 116-118; see EX2020, 75:13-78:19.

Pap.25, 1.

- **Petitioner first argued ligation was accomplished with Linnarsson’s unmodified CDS/TSO.**
- **But Petitioner now argues on Reply for multiple modifications to the CDS/TSO to accomplish ligation.**

Pet.68 (Ground 2, arguing “As Dr. Cooper explains, this approach would be entirely equivalent to the method already disclosed by the combination of Linnarsson and McCloskey, except that instead of having the tag sequence be part of the CDS or TSO prior to extension, the tag sequences are added to the CDS or TSO after extension.”); EX1002 (Cooper Decl.), ¶243.

Rep.24 (arguing for modifications to the biotin and restriction cut sites in the CDS and TSO); EX1059 (Cooper Reply Decl.), ¶¶109-112, 116-118; see EX2020 (Cooper Reply Dep. Tr.), 75:13-78:19; see also SurRep.18-22; Slides 51-52.

Petitioner’s New Arguments Disclaiming Reliance on Mammalian or Other Cells and First Relying, Instead, on Yeast and Bacteria Was Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Mapping to eukaryotic cells (e.g., “tissue of interest,” mouse cells), never mentioning yeast or bacteria, and to CDS with a poly-T sequence to tag mRNA with a poly-A tail. Pet.37-39, 41-42, 44-47; EX1002, ¶¶67, 120-21, 127, 139-41, 143, 153-56, 163-66, 213; see, e.g., EX2020, 59:1-64:2, 71:10-21.	Disclaiming reliance on mammalian cells or other cells—relying instead on yeast, and on bacteria (which, “in general” and as a “rule,” do not have a poly-A tail). Rep.11-13; EX1059, ¶¶86-87; EX1061; EX1063; see, e.g., EX2020, 72:10-75:8.

Pap.25, 1.

- Petitioner first relied on eukaryotic cells and CDS’s with poly-T sequences to tag mRNA with a poly-A tail.
- But Petitioner in Reply now disclaims reliance on mammalian or other cells and relies on yeast and bacteria (which, “in general” and as a “rule,” do not have a poly-A tail).

Pet.37-39, 41-42, 44-47; EX1002 (Cooper Decl.), ¶¶67, 120-21, 127, 139-41, 143, 153-56, 163-66, 213; see, e.g., EX2020 (Cooper Reply Dep. Tr.), 59:1-64:2, 71:10-21; Slide 41.

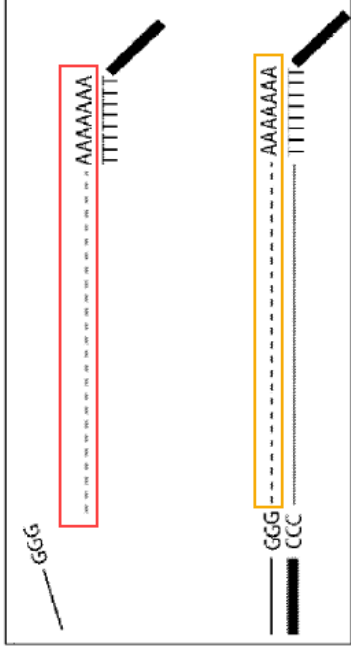
Rep.11-13; EX1059 (Cooper Reply Decl.), ¶¶86-87; EX1061; EX1063; see, e.g., EX2020 (Cooper Reply Dep. Tr.), 72:10-75:8; Slides 38-41.

Petitioner’s New Arguments Disclaiming Reliance on Mammalian or Other Cells and First Relying, Instead, on Yeast and Bacteria Was Beyond the Proper Scope (cont’d)

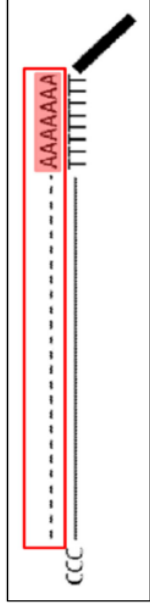
- Examples of Petitioner’s repeated use of eukaryotic mRNA

“In one embodiment, Linnarsson’s method comprises the following steps: ‘(A) the tissue of interest is dissected; (B) *a plurality of single cells are selected*; (C) *single cells are placed in separate wells of a 96-well plate and lysed...*”

Pet.37; Slide 41.

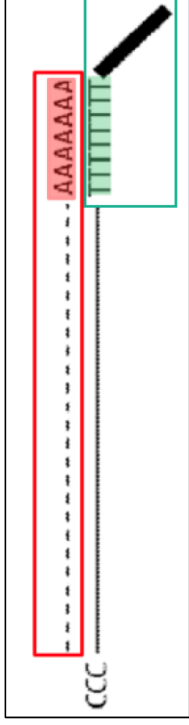


EX 1003 at Fig. 2.

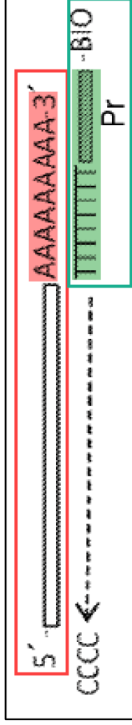


EX1003 at Fig. 2;

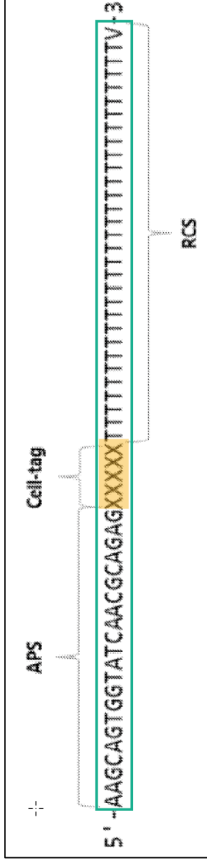
Pet.44-45; Slide 41.



EX1003 at Fig. 2



Id. at Fig. 11



EX1003 at Fig. 4

Pet.41-42; Slide 41.

Petitioner’s New Arguments That Sequencing Limitation is Met by Sequencing Only a Portion of the Tagged Polynucleotide Were Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Sequencing limitation is met by Linnarsson teaching sequencing entirety of the tagged polynucleotide. Pet.56-57; EX1002, ¶198.	Sequencing limitation is met by Linnarsson sequencing only a portion of the tagged polynucleotide. Rep.5; EX1059, ¶¶54-63.

Pap.25, 2.

- Petitioner originally argued the sequencing limitation was met by Linnarsson teaching performing sequencing on the tagged polynucleotide, which Petitioner mapped to the *full length* cDNA generated by reverse transcription.

Pet.56-57; EX1002 (Cooper Decl.), ¶198; SurRep.8-16; Slide 26.
- But Petitioner now argues, on Reply, that the sequencing limitation is met by Linnarsson sequencing only a portion of the tagged polynucleotide.

Rep.5; EX1059 (Cooper Reply Decl.), ¶¶54-63; SurRep.8-16; Slides 31-32.

Petitioner’s New Arguments Changing its Reliance on “Uniquely” Tagging “Each”/“Every”/“Substantially All” mRNA for its Motivation to Combine Were Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Motivation to combine relies on “each”/“every”/“substantially all” mRNA be “uniquely” tagged. Pet.41, 54; EX1002, ¶¶92; EX2022, ¶222.	No requirement that “all the mRNA molecules” be tagged; only need to tag “a subset of molecules” and could accomplish with “gene family-specific” RCS. Rep.12-13; EX1059, ¶¶18, 89.

Pap.25, 2.

- Petitioner originally argued its motivation to combine relies on “each”/“every”/“substantially all” mRNA being “uniquely” tagged.
- But Petitioner now argues on Reply that there is no requirement that “all the mRNA molecules” be tagged.

Pet.41, 54; EX1002 (Cooper Decl.), ¶92; EX2022 (Cooper IPR2023-00955 Decl.), ¶222; SurRep.5-6; Slide 35.

Rep.12-13; EX1059 (Cooper Reply Decl.), ¶¶18, 89; SurRep.5-6; Slide 43.

Petitioner’s New Arguments About When Ligation Occurs Were Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Ligation occurs at some unspecified time “after extension” to the CDS or TSO. EX2018, 54:8-21 (“I <i>did not explicitly say a timing element</i> there...”); EX1002, ¶¶237-244; Pet.66-68.	Ligation occurs “before amplification” and “after the first reverse transcription step.” Rep.17; EX1059, ¶¶113-115.

Pap.25, 2.

- Petitioner first argued ligation occurs at some unspecified time “after extension” to the CDS or TSO.
- But Petitioner now argues on Reply that ligation occurs “before amplification” and “after the first reverse transcription step.”

EX2018 (Cooper Dep. Tr.), 54:8-21 (“I did not explicitly say a timing element there...”); EX1002 (Cooper Decl.), ¶¶237-244; Pet.66-68; see also SurRep.23-24.

Rep.17; EX1059 (Cooper Reply Decl.), ¶¶113-115 (“As clearly discussed in my original declaration, the second tag is ligated to the sample polynucleotide after the first reverse transcription step in Linnarsson’s method.”); see also SurRep.23.

Petitioner’s New Arguments that Linnarsson/McCloskey/Hug Are Analogous to the ‘981 Patent Were Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
No assertion in Petition or Cooper’s original declaration that McCloskey or Linnarsson are analogous to the ‘981 patent (rather than to each other), or that the Petition’s or original declaration’s descriptions showed they were; no assertion about analogousness and Hug. Pet.52; EX1002, ¶187; see Rep.24-25 (no citations to petition).	New assertions that Linnarsson/McCloskey/Hug analogous to ‘981. EX1059, ¶¶124, 127-128; Rep.24-25 & n.6; EX2020, 20:4-28:16, esp. 23:11-19. Also incorporating by reference (Rep.25) Cooper’s arguments he had already provided “detailed obviousness analysis... including explanations of how they are analogous” and in same field “as the ‘981...” in EX1002. EX1059, ¶126; see, e.g., EX2020, 20:4-26:19.

Pap.25, 2.

- The Petition and Dr. Cooper’s original declaration never asserted McCloskey, Linnarsson, or Hug were analogous to the ‘981 patent.
- But Petitioner now asserts on Reply that they are.

Pet.52; EX1002 (Cooper Decl.), ¶187; see Rep.24-25 (no citations to petition); see also SurRep.27-28; Slide 65.

EX1059 (Cooper Reply Decl.), ¶¶124, 127-128; Rep.24-25 & n.6; EX2020 (Cooper Reply Dep. Tr.), 20:4-28:16, esp. 23:11-19; see also SurRep.27-28; Slide 65.

Petitioner’s New Arguments to Modify Hug’s Linker Were Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
Hug (EX1007) was not part of the combination of references making up the Petitioner’s grounds. See Pet.37, 66-69.	“[O]bvious to modify... Hug’s linker molecule to” function as part of Petitioner’s asserted combination of references. EX1059, ¶122 (cited Rep.18). See, e.g., EX2020, 144:9-145:17.

Pap.25, 2.

- Petitioner originally did not argue that Hug (EX1007) was part of the combination of references making up the Petitioner’s grounds.
- But Petitioner now argues on Reply that it would have been “[o]bvious to modify” Hug as a part of the combination of references.

See Pet.37, 66-69; SurRep.23-24.

EX1059 (Cooper Reply Decl.), ¶122 (cited Rep.18); see, e.g., EX2020 (Cooper Reply Dep. Tr.), 144:9-145:17; SurRep.23-24.

Petitioner’s New Argument Relying on EX1065 Was Beyond the Proper Scope

Original Theory/Evidence	New Theory/Evidence
McCloskey is prior art, relying on EX1028. Pet.29-30 (citing EX1028).	McCloskey is prior art, abandoning EX1028 and relying instead on new EX1065. Rep.3 (citing EX1065).

Pap.25, 2.

- Petitioner originally relied on EX1028 to establish McCloskey as prior art.

Pet.29-30; SurRep.25-27; Slide 66.

- But on Reply Petitioner abandoned EX1028 and replaced it with a Declaration (EX1065) newly submitted with its Reply to establish McCloskey as prior art.

Rep.3 (citing EX1065); SurRep.25-27; Slide 67.

Dr. Cooper's Testimony Should Be Given Little to No Weight



Dr. Cooper's Opinions Are Inconsistent and Should Be Given Little to No Weight

- Dr. Cooper claimed at deposition he had “repeatedly” asserted in his opening declaration (EX1002) that it would have been obvious to expand McCloskey’s barcodes to different lengths, based on his citation to a page of McCloskey that “includes the 4n paragraph – which includes the 4n definition for the barcode.” Bet Dr. Cooper could not find *even a single mention* of this “4n language” in that original declaration (EX1002).
- Dr. Cooper also admitted at deposition that his ligation opinions “did not explicitly say a timing element” (EX2018 (Cooper Dep. Tr.), 54:8-21) but then in his declaration in an IPR on a related patent with near-verbatim language (EX1059, ¶113) testified “[a]s clearly discussed in my original declaration, the second tag is ligated... after the first reverse transcription step....”

EX2020 (Cooper Reply Dep. Tr.), 36:19-58:1; EX2018 (Cooper Dep. Tr.), 54:8-21; EX2013 (Quackenbush Decl.), ¶106; SurRep.25.

SurRep.25; *Applied Materials, Inc. v. Demaray LLC*, IPR2021-00104, Pap.54, 29-33 (June 29, 2022) (rejecting petitioner’s reliance on expert’s testimony that was “belled by [the expert’s] own testimony”).

Dr. Cooper Failed to Consider Readily-Available Evidence When Making His Sweeping And Demonstrably False Assertions

- Dr. Cooper admitted that “people of skill at the time of the ’981 patent had resources available to identify restriction[] cut sites.”

EX2020 (Cooper Reply Dep. Tr.), 120:20-121:16; SurRep.10; see Slide 14.

- Dr. Cooper admitted he “did not” “look for any other cut site” other than “the cut site that is referred to in Figure 11.”

EX2020 (Cooper Reply Dep. Tr.), 120:7-18, 137:7-10; SurRep.9-10; see Slide 14.

- Yet Dr. Cooper testified that “Figures 3 and 4 do not include... *a restriction enzyme cut site at all....*”

E.g., EX1059 (Cooper Reply Decl.), ¶¶118, 42, 66-67 (testifying Figures 3 and 4 “do not include... any restriction enzyme cut site”); SurRep.9-10; see Slide 13.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of PATENT OWNER'S 10X GENOMICS, INC.'S DEMONSTRATIVE SLIDES has been served in its entirety by causing the aforementioned document to be electronically mailed to the following attorneys of record for the Petitioner listed below:

Petitioner's Counsel of Record:

Lead Counsel:	Derek C. Walter Reg. No. 74,656 derek.walter@weil.com Weil, Gotshal & Manges LLP 201 Redwood Shores Parkway Redwood Shores, CA 94065 Telephone: (650) 802-3934 By Electronic Mail
Backup Counsel:	Adrian C. Percer Registration No. 46,986 adrian.percer@weil.com Weil, Gotshal & Manges LLP 201 Redwood Shores Parkway Redwood Shores, CA 94065 Telephone: (650) 802-3124 William Sutton Ansley Registration No. 67,828 sutton.ansley@weil.com Weil, Gotshal & Manges LLP 2001 M Street NW, Suite 600 Washington, DC 20036 Telephone: (202) 682-7018 By Electronic Mail Edward R. Reines (<i>pro hac vice</i>) edward.reines@weil.com Weil, Gotshal & Manges LLP 201 Redwood Shores Parkway Redwood Shores, CA 94065

	Telephone: (650) 802-3022 By Electronic Mail
--	---

Dated: July 19, 2024

Respectfully submitted,

By: /Sayem Osman/
Sayem Osman