



ORAL ARGUMENT FOR IPR2025-00015 IPR2025-00016

*U.S. Patent Nos.
10,036,012 and 10,876,108*

Patent Owner's Hearing Demonstratives

January 22, 2026

Key Points

➤ **Meyer** does not anticipate claim 1 of the '012 and '108 patents

- Challenged claims require enrichment
- Meyer does not disclose enrichment of any sequences targeted by the target-specific adaptors

➤ **Meyer** does not anticipate claim 21 of the '012 patent

- Claim 21 requires that the claimed nucleic acid fragment be obtained by reverse transcribing an RNA fragment
- Meyer does not disclose fragmentation of RNA prior to reverse transcription

➤ **Meyer** does not anticipate claim 22 of the '012 patent

- Meyer does not disclose adaptors ligated to both ends of Petitioner's target nucleic acid fragments

➤ Petitioner has failed to show that any challenged claims are obvious

Key Points

Meyer does not anticipate claim 1 of the '012 and '108 patents

- Challenged claims require enrichment
- Meyer does not disclose enrichment of any sequences targeted by the target-specific adaptors

Meyer does not anticipate claim 21 of the '012 patent

- Claim 21 requires that the claimed nucleic acid fragment be obtained by reverse transcribing an RNA fragment
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Meyer does not anticipate claim 22 of the '012 patent

- Meyer does not disclose adaptors ligated to both ends of Petitioner's target nucleic acid fragments

 Petitioner has failed to show that any challenged claims are obvious

The Petition Suffers Three Fatal Flaws

Petitioner Argues:

1

Enrichment is not based on proportionality

2

The *Meyer* method enriched for the 3' fragment

3

A POSA would infer enrichment from *Meyer's* reported data

The Claims Require That the “Amplifying” Step Result in Enrichment

'012 patent, claim 1

1. A method for sequencing an **enriched** nucleic acid sequence of interest, the method comprising:

* * *

- d) **amplifying the one or more oligonucleotide extension products** using a first primer that anneals to a complement of the first adaptor sequence and a second primer that anneals at its 3' end to a complement of the second adaptor sequence **to enrich for the nucleic acid sequence of interest**, wherein products of the amplifying comprise a 3' end with sequence complementary to a sequence on a surface;

* * *

- f) sequencing **the enriched nucleic acid sequence of interest** on a massively parallel sequencing platform.

'108 patent, claim 1

1. A method for sequencing an **enriched** nucleic acid sequence of interest, the method comprising:

* * *

- c) **amplifying the one or more oligonucleotide extension products**, in the reaction mixture, using a first primer that anneals to the complement of the first adaptor sequence and a second primer that anneals at its 3' end to a complement of the second adaptor sequence, **thereby enriching the nucleic acid sequence of interest**, by generating amplified products comprising the enriched nucleic acid sequence of interest; and
- d) sequencing the amplified products comprising **the enriched nucleic acid sequence of interest** on a massively parallel sequencing platform.

Proposed Constructions

Term	Tecan's Construction	Petitioner's Construction
“amplifying the one or more oligonucleotide extension products . . . [to enrich for/thereby enriching] the nucleic acid sequence of interest”	“increase the proportion of the nucleic acid sequence of interest relative to the nucleic acid fragments in the solution not containing the sequence of interest by amplifying the oligonucleotide extension product and its complement” Paper 41, 21.	No express construction offered. “increase in the sequence of interest over the baseline” raw number value “if the method were not performed” Paper 47, 6-8.

The Challenged Claims Are Directed to an Enrichment Method

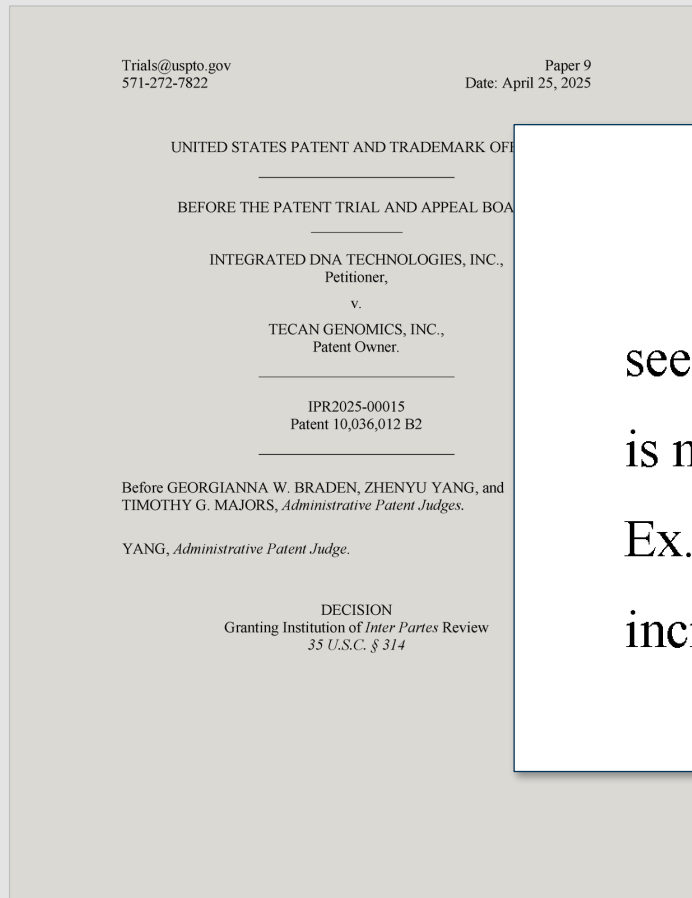
 US010036012B2		
(12) United States Patent Amorese et al.	(10) Patent No.: US 10,036,012 B2	
	(45) Date of Patent: *Jul. 31, 2018	
(54) COMPOSITIONS AND METHODS FOR TARGETED NUCLEIC ACID SEQUENCE ENRICHMENT AND HIGH EFFICIENCY LIBRARY GENERATION	5,171,534 A 12/1992 Smith et al.	
(71) Applicant: NuGEN Technologies, Inc., San Carlos, CA (US)	5,194,370 A 3/1993 Berninger et al.	
(72) Inventors: Doug Amorese, Los Altos, CA (US); Chris Armour, Kirkland, WA (US); Nurith Keren, Palo Alto, CA (US)	5,216,141 A 6/1993 Boerner	
(73) Assignee: NUGEN TECHNOLOGIES, INC., San Carlos, CA (US)	5,234,809 A 8/1993 Bism et al.	
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.	5,235,033 A 8/1993 Summerton et al.	
This patent is subject to a terminal disclaimer.	5,242,794 A 9/1993 Whitley et al.	
(21) Appl. No.: 15/471,785	5,380,823 A 1/1995 Saugli et al.	
(22) Filed: Mar. 28, 2017	5,399,491 A 3/1995 Kacian et al.	
(65) Prior Publication Data US 2017/0298345 A1 Oct. 19, 2017	5,409,818 A 4/1995 Davey et al.	
Related U.S. Application Data	5,418,149 A 5/1995 Gelfand et al.	
(63) Continuation of application No. 13/750,768, filed on Jun. 25, 2013, now Pat. No. 8,650,628.	5,427,929 A 6/1995 Richards et al.	
(64) Provisional application No. 61/591,241, filed on Jan. 26, 2012.	5,480,784 A 1/1996 Kacian et al.	
(51) Int. Cl. C12N 15/10 (2006.01)	5,494,810 A 2/1996 Barany et al.	
C12N 15/66 (2006.01)	5,508,169 A 4/1996 Douglas et al.	
(52) U.S. Cl. C12N 15/066 (2013.01); C12N 15/072 (2013.01); C12N 15/66 (2013.01); C12Q 2525/191 (2013.01)	5,508,178 A 4/1996 Rose et al.	
(58) Field of Classification Search None See application file for complete search history.	5,510,720 A 4/1996 Foster et al.	
(56) References Cited	5,525,471 A 6/1996 Zeng	
U.S. PATENT DOCUMENTS	5,545,522 A 8/1996 Via Gelder et al.	
4,362,867 A 12/1982 Paddock	5,554,516 A 9/1996 Kacian et al.	
4,458,066 A 7/1984 Canuthers et al.	5,554,517 A 9/1996 Davey et al.	
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4,676,187 A 10/1989 Deck et al.	5,573,813 A 11/1996 Rosenmeyer et al.	
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4,942,124 A 7/1990 Church	5,589,339 A 12/1996 Hampton et al.	
4,988,617 A 1/1991 Landegren et al.	(Continued)	
4,996,143 A 2/1991 Heller et al.		
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5,043,272 A 8/1991 Hartley		
5,082,830 A 1/1992 Baskel et al.		
5,090,591 A 2/1992 Long		
5,130,238 A 7/1992 Malick et al.		
5,169,766 A 12/1992 Schuster et al.		

(57) ABSTRACT

The present invention provides methods, compositions and kits for targeted nucleic acid sequence enrichment in a nucleic acid sample and for high efficiency nucleic acid library generation for next generation sequencing (NGS). Specifically, the methods, compositions and kits provided herein are useful for the production and capture of amplification-ready, target-specific and strand-specific regions of interest from nucleic acid samples containing complex DNA.

EX1001, Abstract

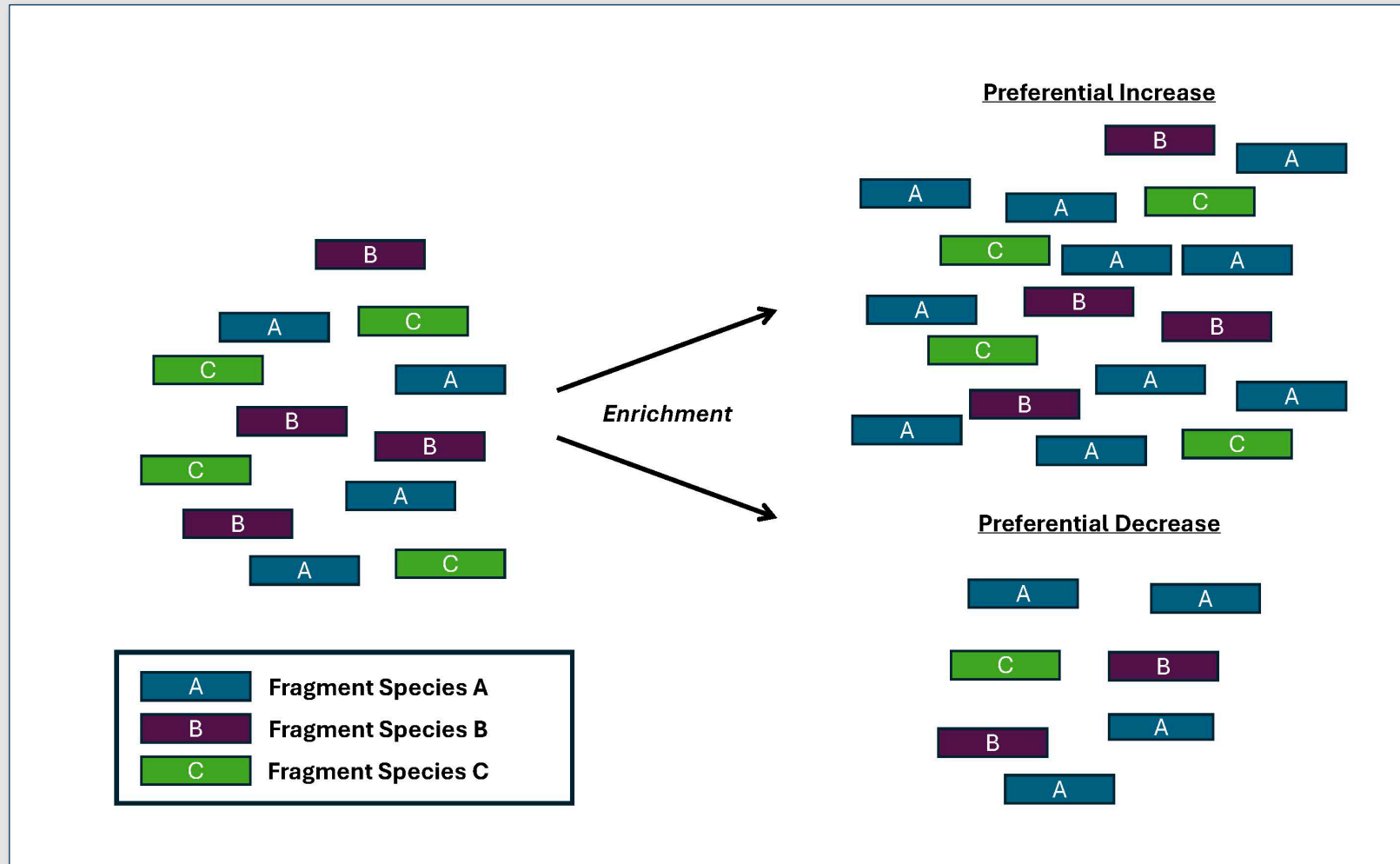
Tecan's Interpretation of "Enrich" Is Consistent With the Board's Adoption of the Plain and Ordinary Meaning of the Term



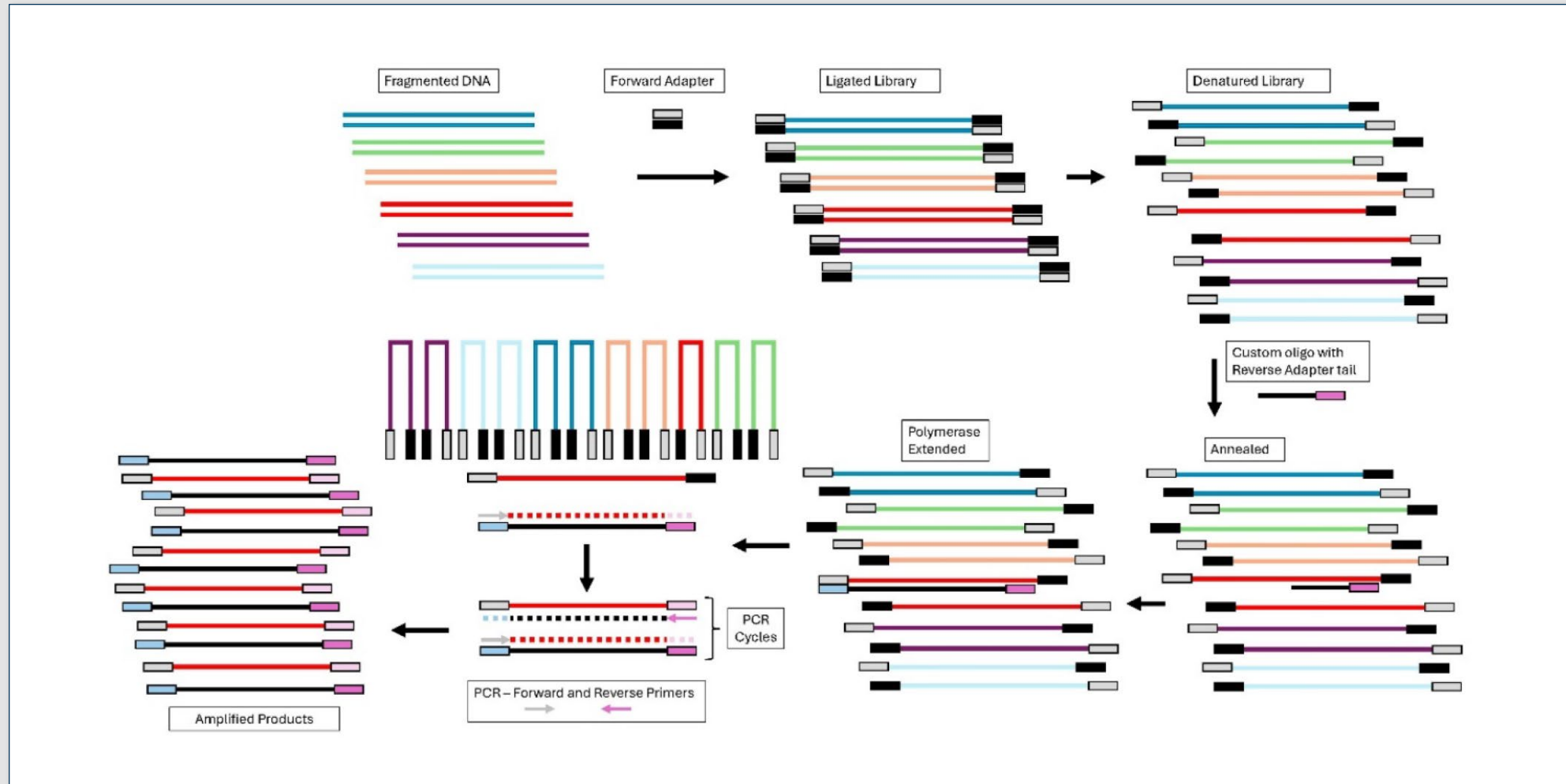
Neither party proposes any special meaning for the term “enrich.” We see no reason to do so either. To the extent express construction of “enrich” is necessary, we adopt the plain and ordinary meaning of the term. *See* Ex. 3001 (entry e defining “enrich” to mean “to process so as to add or increase the proportion of a desirable ingredient”).

Paper 9, 15

Enrichment Requires a Change in Relative Abundance



Tecan's Interpretation Is Consistent With the Intrinsic Record



IPR2025-00015: Paper 41, 11-16; Paper 53, 9; IPR2025-00016: Paper 39, 11-15; Paper 51, 9; see *also* EX2038. ¶¶121-131

Dr. Sims' Testimony Supports Tecan's Interpretation

situations where a -- an enrichment process results in overrepresentation of certain nucleic acid sequences **relative to others** in a sample.

EX2037.0173:15-17

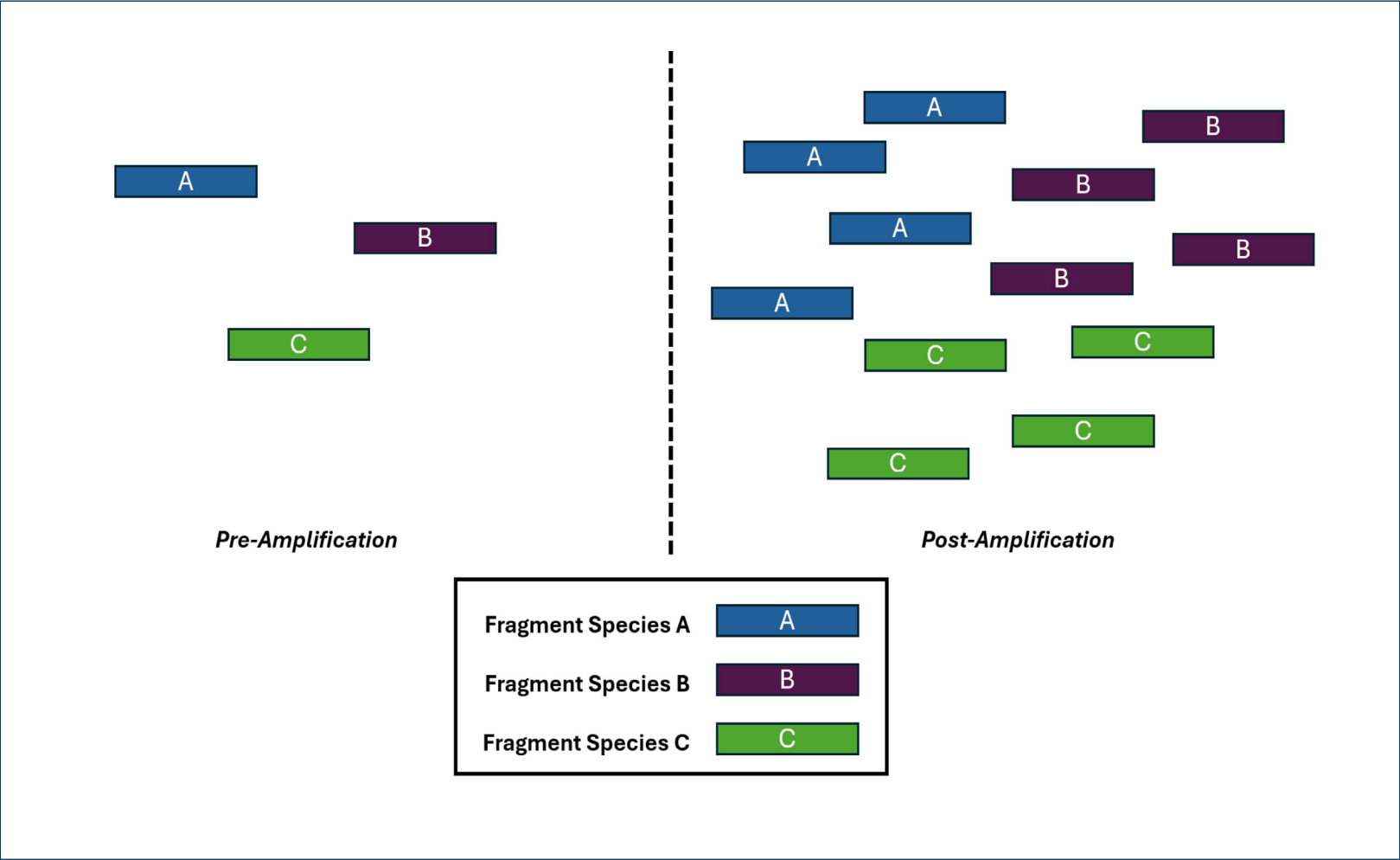
enrichment, but my interpretation is that "enriched" in this context refers to overrepresentation of certain nucleic acids in a library **relative to others.**

EX2037.0150:20-22

A. To assess enrichment, one would **compare the abundance of sequences of interest to that of other sequences in the sample.**

EX2037.0217:15-17

Petitioner's Proposal Reads Out the Enrichment Requirement



Enrichment Is Material to Patentability

'012 patent, claim 1

1. A method for sequencing an **enriched** nucleic acid sequence of interest, the method comprising:

* * *

d) **amplifying the one or more oligonucleotide extension products** using a first primer that anneals to a complement of the first adaptor sequence and a second primer that anneals at its 3' end to a complement of the second adaptor sequence **to enrich for the nucleic acid sequence of interest**, wherein products of the amplifying comprise a 3' end with sequence complementary to a sequence on a surface;

* * *

f) sequencing **the enriched nucleic acid sequence of interest** on a massively parallel sequencing platform.

'108 patent, claim 1

1. A method for sequencing an **enriched** nucleic acid sequence of interest, the method comprising:

* * *

c) **amplifying the one or more oligonucleotide extension products**, in the reaction mixture, using a first primer that anneals to the complement of the first adaptor sequence and a second primer that anneals at its 3' end to a complement of the second adaptor sequence, **thereby enriching the nucleic acid sequence of interest**, by generating amplified products comprising the enriched nucleic acid sequence of interest; and

d) sequencing the amplified products comprising **the enriched nucleic acid sequence of interest** on a massively parallel sequencing platform.

Enrichment Is Material to Patentability



when a “whereby” or [similar] clause states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention.

Allergan Sales, LLC v. Sandoz, Inc., 935 F.3d 1370, 1376 (Fed. Cir. 2019) (cleaned up)

There is a need for improved methods for selective target enrichment that allow for low-cost, high throughput capture

* * *

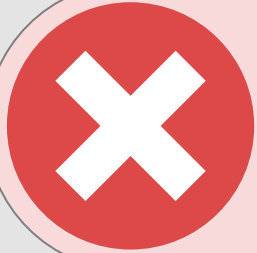
The methods of the invention can be used for the selective enrichment of a plurality of defined target sequences from complex DNA with a set of common primers and adaptors,

* * *

reference sequences. Fold enrichment is determined by calculating the number of rRNA reads as a percentage of total mapped reads in the control and test samples.

EX1001.0016 (2:25-28), 18 (6:12-16), 31 (31:41-32:42)

The Petition Suffers Three Fatal Flaws

-  Enrichment is not based on proportionality
 -  Claimed enrichment requires increase in proportion
- 2 The *Meyer* method enriched for the 3' fragment
 - 3 A POSA would infer enrichment from *Meyer's* reported data

Dr. Meyer Testified That the *Meyer* Method Did Not Involve Target Sequence Enrichment

70. Our study was not designed to target specific regions of the mRNA transcripts. In order to accurately sequence the entire length of the transcripts, we specifically did not want to enrich certain targets within those transcripts.

EX2287.¶70

Q. Did this experiment involve enriching for any particular sequences?

A. We were not enriching for any particular sequence in terms of particular genes or families of genes. We were enriching for expressed genes; that is, mRNA as compared to any of the other nucleic acids in an organism.

EX1126.0030:6-13 (Meyer Tr.)

EX2287.¶77

77. Any suggestion that the data reflects an enrichment of the 3' end fragments over the other cDNA fragments in our library of fragments that were sequenced misinterprets what we actually reported in Meyer.

Meyer Method – Figure 1

Research article

Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFLx

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Email: Eli Meyer^{*} - ElMeyer@mail.utexas.edu; Galina V Aglyamova - aglyamova@mail.utexas.edu; Shi Wang - swang@mail.utexas.edu; Jade Buchanan-Carter - jabuchan@indiana.edu; David Abrego - david.abrego@jcu.edu.au; John K Colbourne - jcolbour@cgb.indiana.edu; Bette L Willis - bette.willis@jcu.edu.au; Mikhail V Matz - matz@mail.utexas.edu

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This article is available from: <http://www.biomedcentral.com/1471-2164/10/219>

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Abstract

Background: New methods are needed for genomic-scale analysis of emerging model organisms that exemplify important biological questions but lack fully sequenced genomes. For example, there is an urgent need to understand the potential for corals to adapt to climate change, but few molecular resources are available for studying these processes in reef-building corals. To facilitate genomics studies in corals and other non-model systems, we describe methods for transcriptome sequencing using 454, as well as strategies for assembling a useful catalog of genes from the output. We have applied these methods to sequence the transcriptome of planulae larvae from the coral *Acropora millepora*.

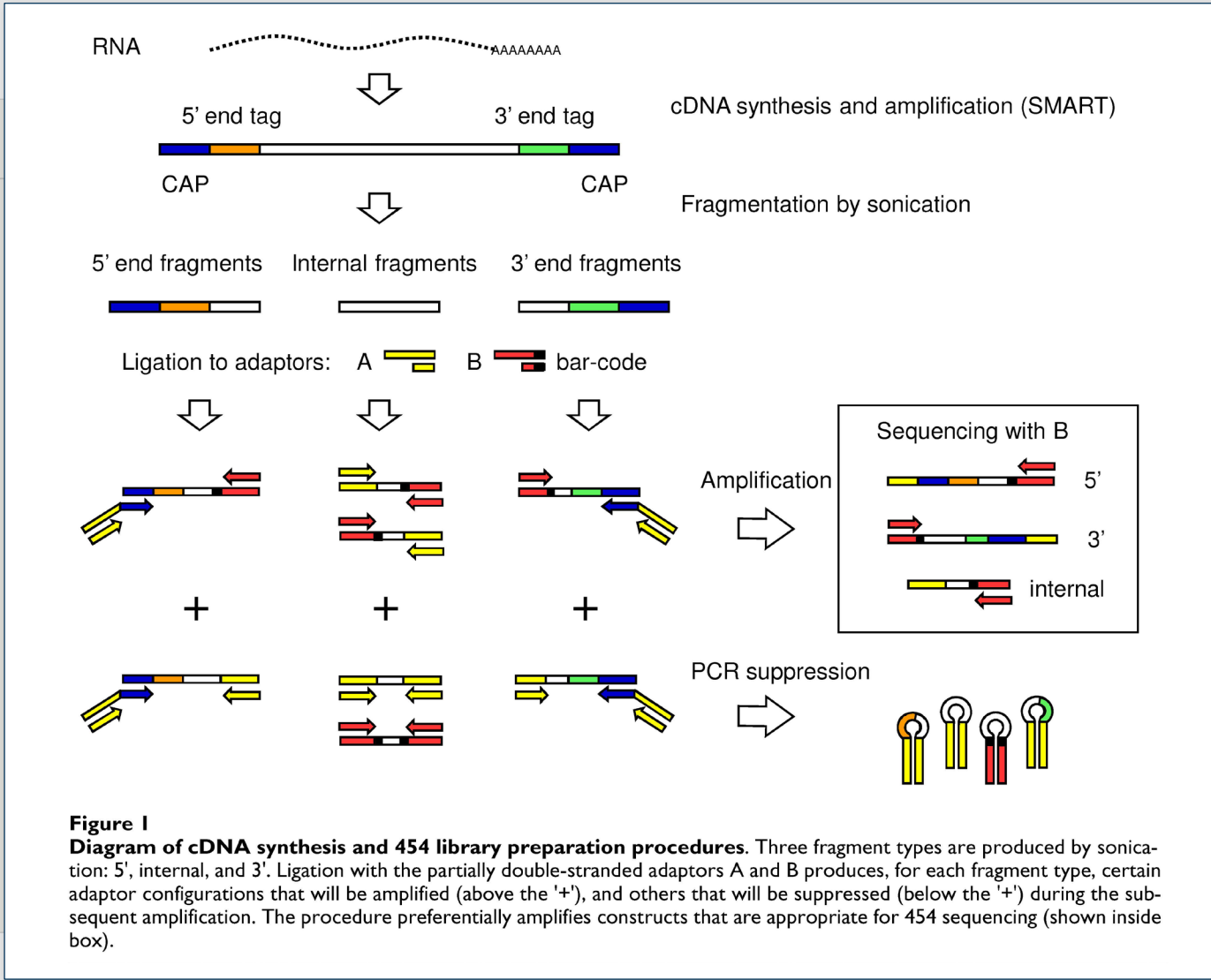
Results: More than 600,000 reads produced in a single 454 sequencing run were assembled into ~40,000 contigs with five-fold average sequencing coverage. Based on sequence similarity with known proteins, these analyses identified ~11,000 different genes expressed in a range of conditions including thermal stress and settlement induction. Assembled sequences were annotated with gene names, conserved domains, and Gene Ontology terms. Targeted searches using these annotations identified the majority of genes associated with essential metabolic pathways and conserved signaling pathways, as well as novel candidate genes for stress-related processes. Comparisons with the genome of the anemone *Nematostella vectensis* revealed ~8,500 pairs of orthologs and ~100 candidate coral-specific genes. More than 30,000 SNPs were detected in the coral sequences, and a subset of these validated by re-sequencing.

Conclusion: The methods described here for deep sequencing of the transcriptome should be widely applicable to generate catalogs of genes and genetic markers in emerging model organisms. Our data provide the most comprehensive sequence resource currently available for reef-building corals, and include an extensive collection of potential genetic markers for association and population connectivity studies. The characterization of the larval transcriptome for this widely-studied coral will enable research into the biological processes underlying stress responses in corals and evolutionary adaptation to global climate change.

IDT Exhibit 1006.0001

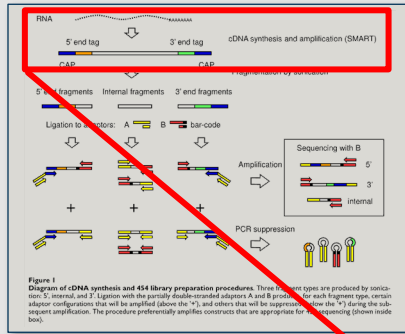
Page 1 of 18

(page number not for citation purposes)



EX1006.0003

Meyer Method – Modified SMART Protocol



The purpose of the “broken chain” T-primer is to reduce read artifacts during 454 pyrosequencing, which may get thrown out of calibration by too strong of a light signal produced from a long mononucleotide stretch (such as polyT or polyA).

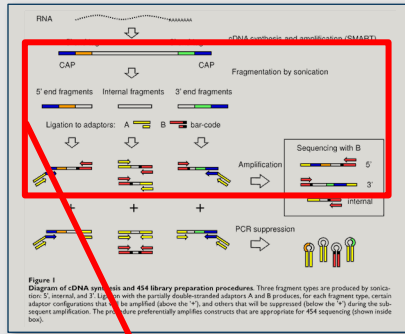
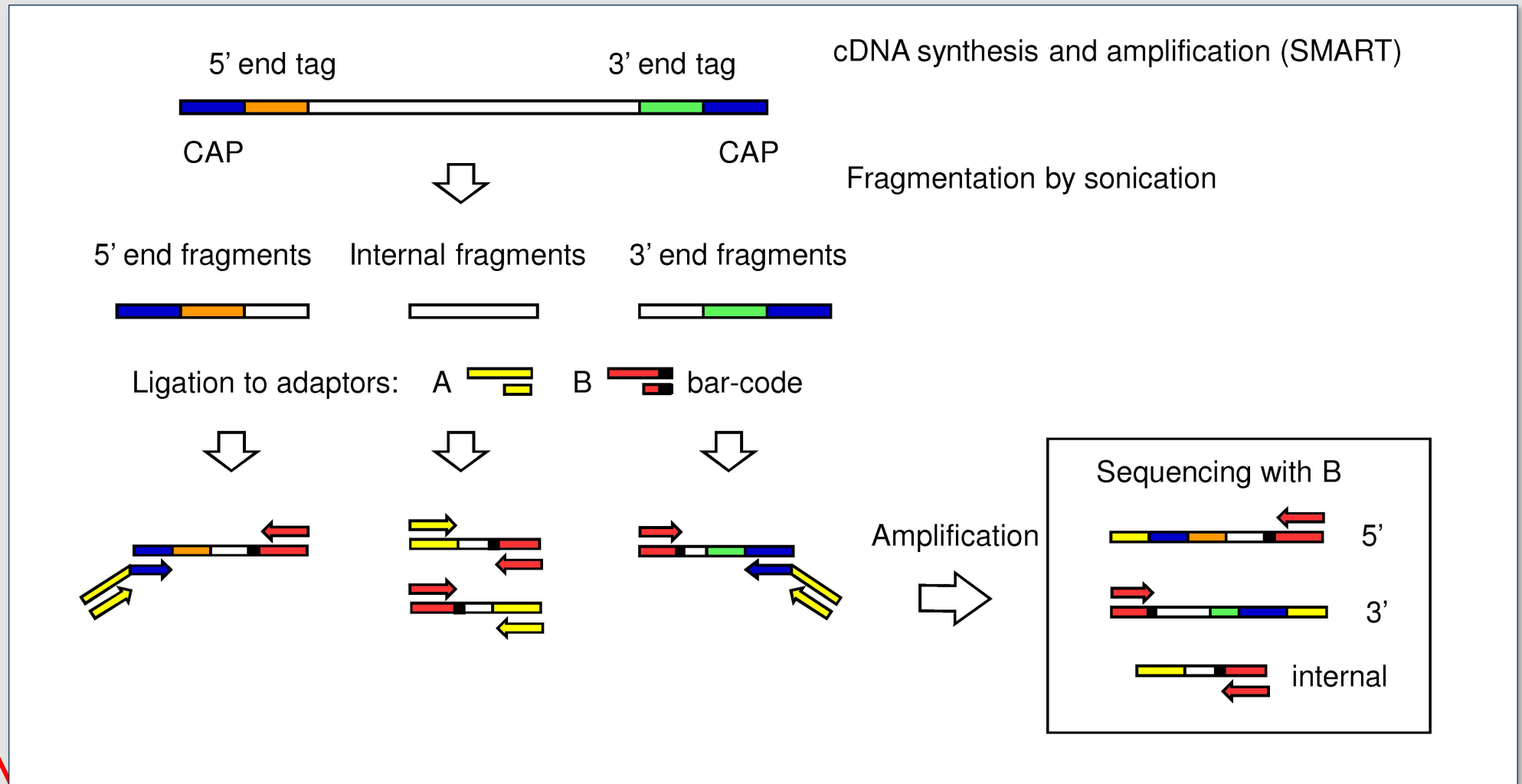
EX1006.0003

EX1006.0019

EX1034.0004

first-strand reaction. However, because RT cannot always transcribe the entire mRNA sequence, the 5' ends of genes tend to be under-represented in cDNA populations. This is often the case for long mRNAs, especially if the first-strand synthesis is primed only with oligo(dT) primers, or if the mRNA has a persistent secondary structure. In the absence of RNA degradation, truncated cDNA molecules present in libraries are often due to RT pausing before transcription is complete. Regardless, the SMART method is able to preferentially enrich for full-length cDNAs.

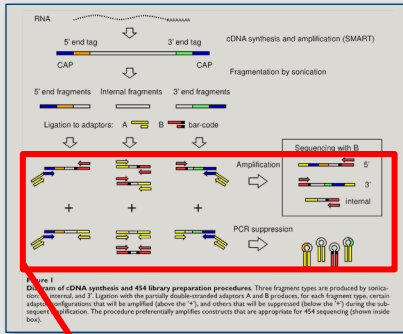
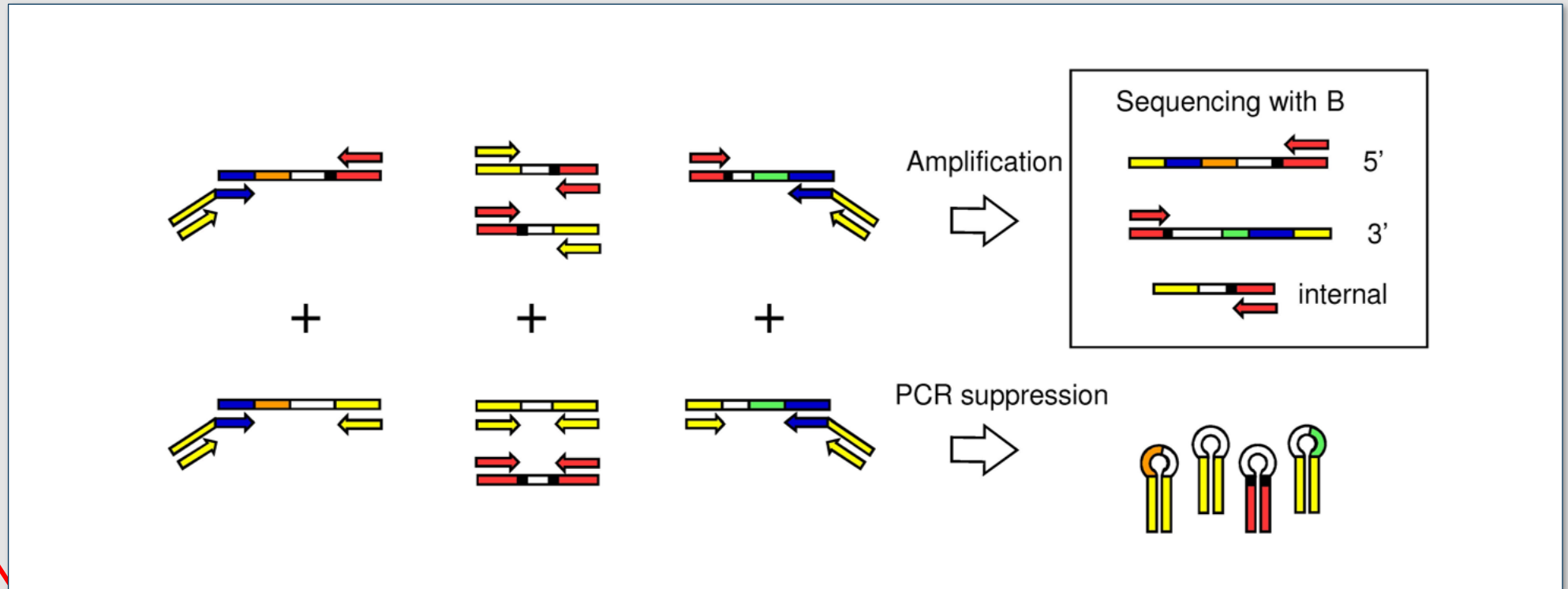
Meyer Method – cDNA Fragmentation, Ligation, Amplification



EX1006.0003

IPR2025-00015: Paper 41, 30-35; IPR2025-00016: Paper 39, 26-31; see also EX2038.¶¶171-184; EX2287.¶¶33-46

Meyer Method – PCR Suppression



EX1006.0003

Meyer Modified Method – Additional File 4

Figure 1 Protocol (EX1006.0003)

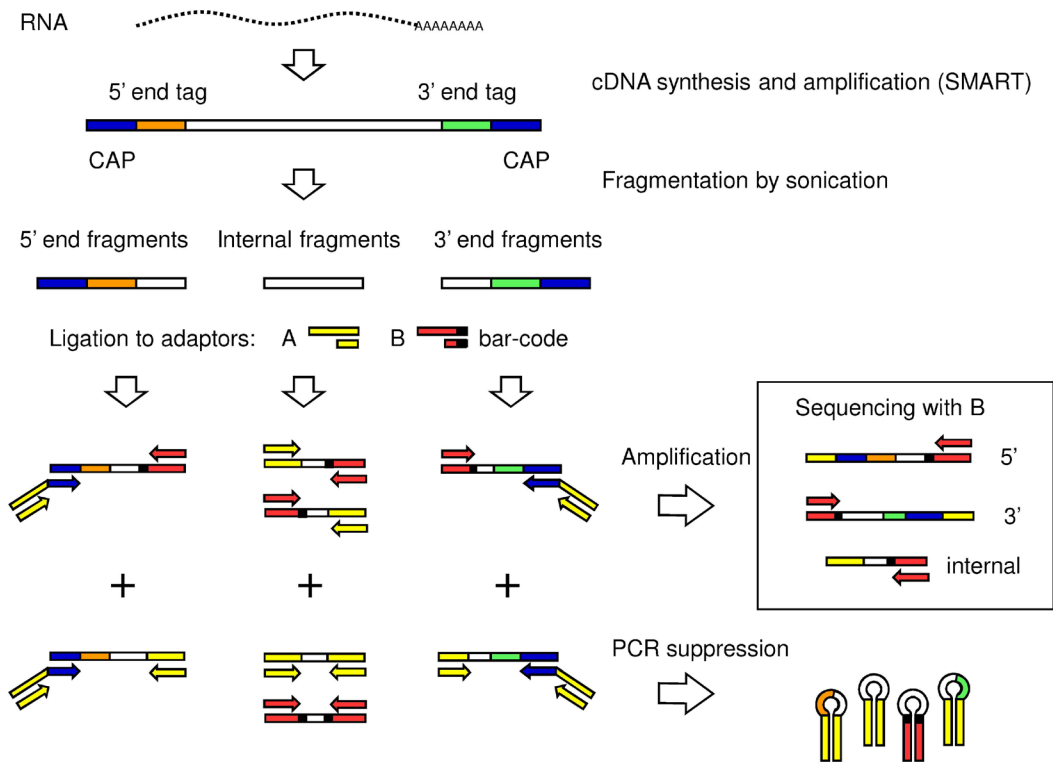
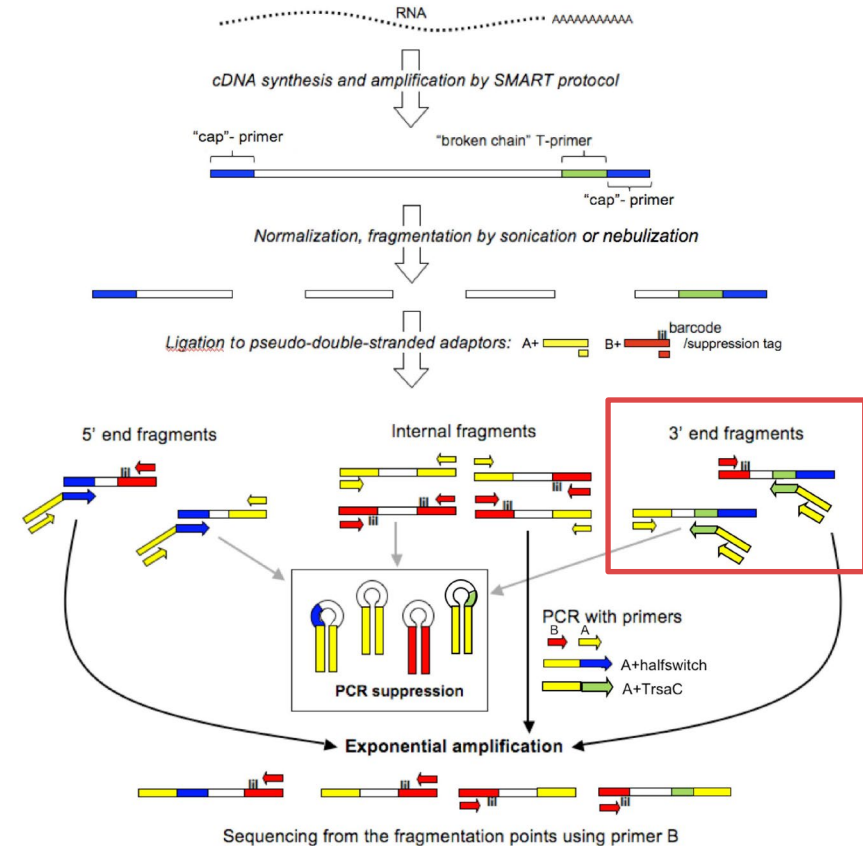


Figure 1
Diagram of cDNA synthesis and 454 library preparation procedures. Three fragment types are produced by sonication: 5', internal, and 3'. Ligation with the partially double-stranded adaptors A and B produces, for each fragment type, certain adaptor configurations that will be amplified (above the '+'), and others that will be suppressed (below the '+') during the subsequent amplification. The procedure preferentially amplifies constructs that are appropriate for 454 sequencing (shown inside box).

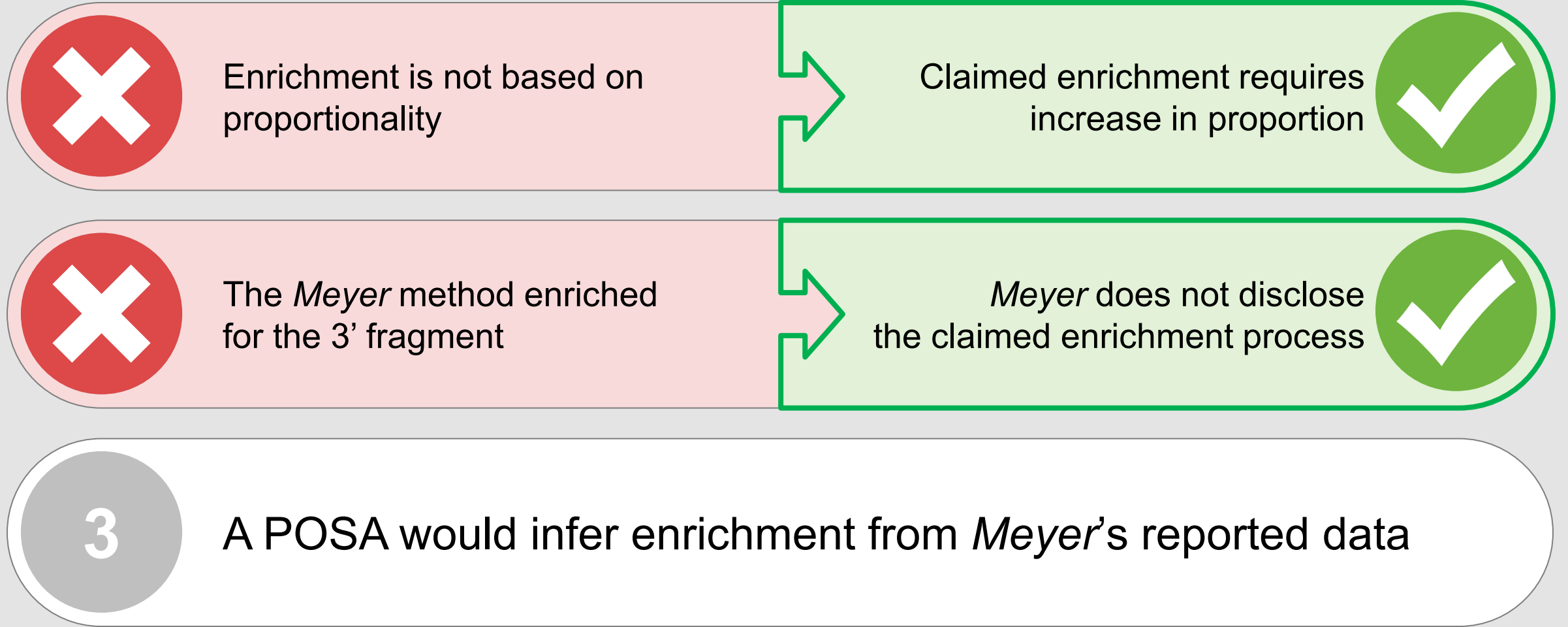
Additional File 4 Protocol (EX1006.0020)



Petitioner's Evolving Enrichment Arguments

Original	<i>Meyer</i> enriches for end fragments
New	<i>Meyer</i> enriches because amount of 3' end fragments increased
New	<i>Meyer</i> enriches over other nucleic acids present in the original sample
New	<i>Meyer</i> anticipates because the patent allows for targeting of a whole transcriptome

The Petition Suffers Three Fatal Flaws



BMC Genomics

BioMed Central

Research article

Sequencing and de novo analysis of a coral larval transcriptome

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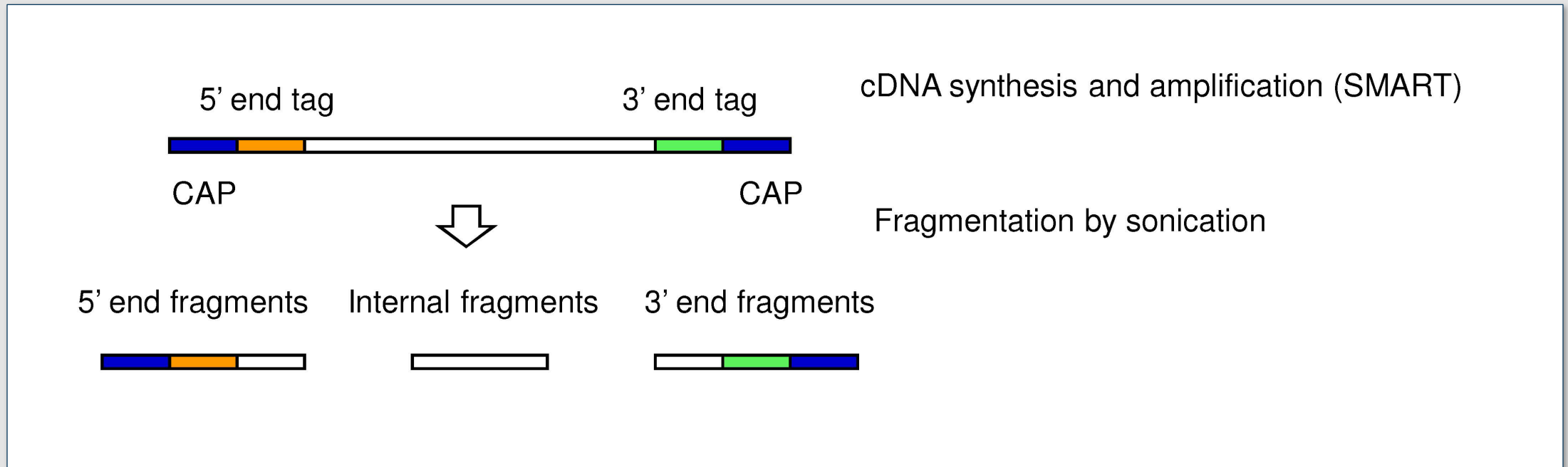
Citation: Guffee D, Shepherd D, Aglyamova CV, Wang S, Buchanan-Carter J, Shih H, Colbourne JR, Weller IU and Kane MV (2013) Sequencing and de novo analysis of a coral larval transcriptome. *BMC Genomics* 14:128. <https://doi.org/10.1186/1471-2164-14-128>

with a single C. A single copy of this 'broken-T' adaptor was found in 98,276 of the raw reads (16% of the total), slightly more than the expectation ($\sim 10\%$) based on average read length (232 bp) and assuming an average transcript size of 2,200 bp.

Meyer's Expectation:

$$\mathbf{10\%} \approx \frac{232 \text{ bp average read length}}{2,200 \text{ bp average transcript length}} \times 100\%$$

Meyer Attempts to Estimate Proportion of 3' Fragments



EX1006.0003

Meyer's Numerator Is Based on Skewed Data

BMC Genomics

Research article

Open Access

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Abstract

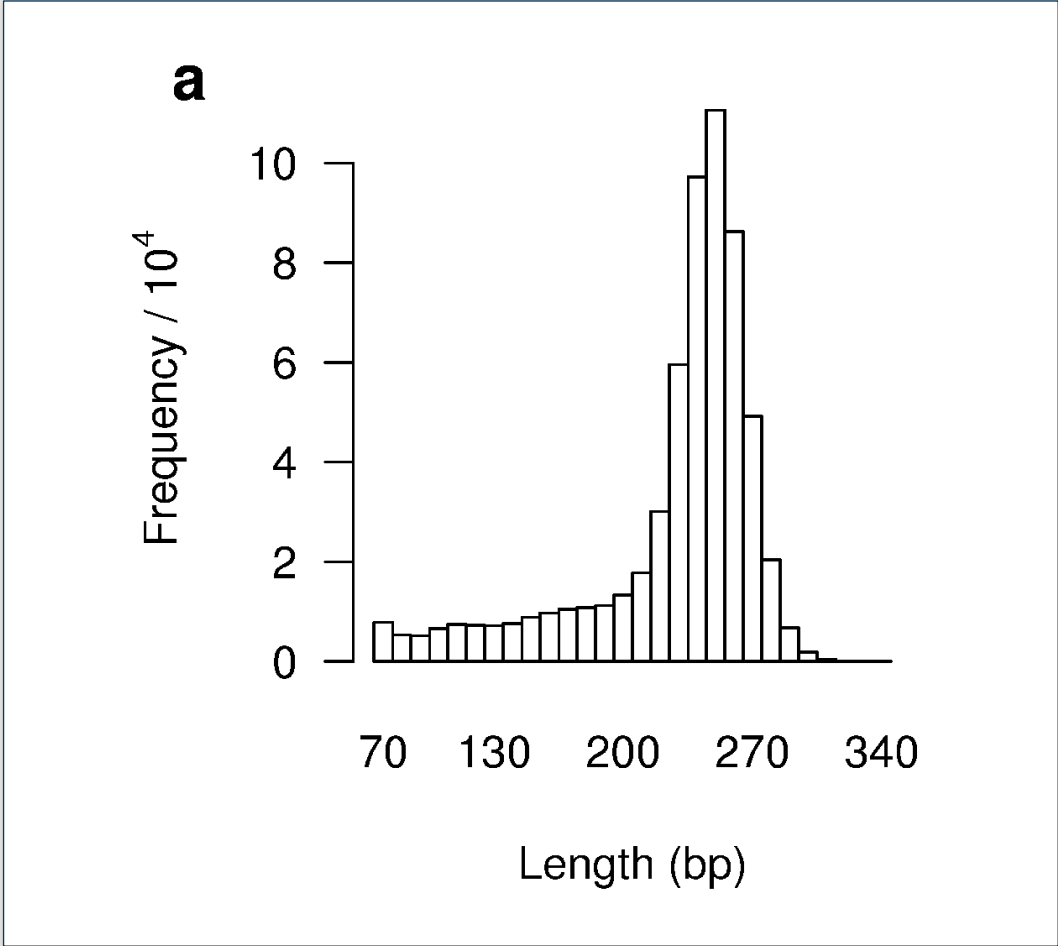
Background: New methods are needed for genomic-scale analysis of emerging model organisms that exemplify important biological questions but lack fully sequenced genomes. For example, there is an urgent need to understand the potential for corals to adapt to climate change, but few molecular resources are available for studying these processes in reef-building corals. To facilitate genomics studies in corals and other non-model systems, we describe methods for transcriptome sequencing using 454, as well as strategies for assembling a useful catalog of genes from the output. We have applied these methods to sequence the transcriptome of planulae larvae from the coral *Acropora millepora*.

Results: More than 600,000 reads produced in a single 454 sequencing run were assembled into ~40,000 contigs with five-fold average sequencing coverage. Based on sequence similarity with known proteins, these analyses identified ~1,000 different genes expressed in a range of conditions including thermal stress and settlement induction. Assembled sequences were annotated with gene names, conserved domains, and Gene Ontology terms. Targeted searches using these annotations identified the majority of genes associated with essential metabolic pathways and conserved signaling pathways, as well as novel candidate genes for stress-related processes. Comparisons with the genome of the anemone *Nematostella vectensis* revealed ~8,500 pairs of orthologs and ~100 candidate coral-specific genes. More than 30,000 SNPs were detected in the coral sequences, and a subset of these validated by re-sequencing.

Conclusion: The methods described here for deep sequencing of the transcriptome should be widely applicable to generate catalogs of genes and genetic markers in emerging model organisms. Our data provide the most comprehensive sequence resource currently available for reef-building corals, and include an extensive collection of potential genetic markers for association and population connectivity studies. The characterization of the larval transcriptome for this widely-studied coral will enable research into the biological processes underlying stress responses in corals and evolutionary adaptation to global climate change.

IDT Exhibit 1006.0001

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(page number not for citation purposes)



EX1006.0004 (Figure 2(a))

IPR2025-00015: Paper 41, 41-42; Paper 53, 18; IPR2025-00016: Paper 39, 37-38; Paper 51, 18; see also EX2038.¶¶254-259

Denominator Estimate: The Reassembled Transcript Lengths Are Skewed

BMC Genomics



Research article

Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFLx

Eli Meyer*¹, Galina V Aglyamova¹, Shi Wang¹, Jade Buchanan-Carter², David Abrego³, John K Colbourne², Bette L Willis³ and Mikhail V Matz¹

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Abstract

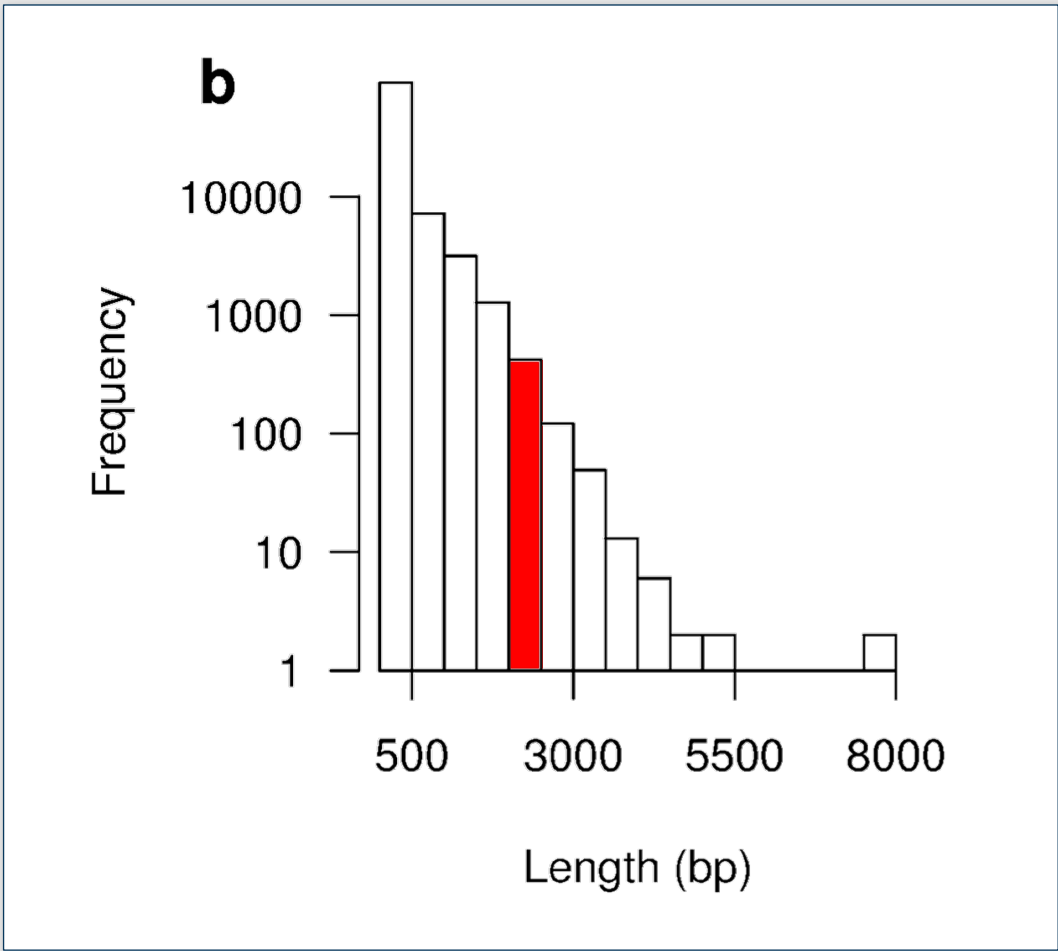
Background: New methods are needed for genomic-scale analysis of emerging model organisms that exemplify important biological questions but lack fully sequenced genomes. For example, there is an urgent need to understand the potential for corals to adapt to climate change, but few molecular resources are available for studying these processes in reef-building corals. To facilitate genomics studies in corals and other non-model systems, we describe methods for transcriptome sequencing using 454, as well as strategies for assembling a useful catalog of genes from the output. We have applied these methods to sequence the transcriptome of planulae larvae from the coral *Acropora millepora*.

Results: More than 600,000 reads produced in a single 454 sequencing run were assembled into ~40,000 contigs with five-fold average sequencing coverage. Based on sequence similarity with known proteins, these analyses identified ~11,000 different genes expressed in a range of conditions including thermal stress and settlement induction. Assembled sequences were annotated with gene names, conserved domains, and Gene Ontology terms. Targeted searches using these annotations identified the majority of genes associated with essential metabolic pathways and conserved signaling pathways, as well as novel candidate genes for stress-related processes. Comparisons with the genome of the anemone *Nematostella vectensis* revealed ~8,500 pairs of orthologs and ~100 candidate coral-specific genes. More than 30,000 SNPs were detected in the coral sequences, and a subset of these validated by re-sequencing.

Conclusion: The methods described here for deep sequencing of the transcriptome should be widely applicable to generate catalogs of genes and genetic markers in emerging model organisms. Our data provide the most comprehensive sequence resource currently available for reef-building corals, and include an extensive collection of potential genetic markers for association and population connectivity studies. The characterization of the larval transcriptome for this widely-studied coral will enable research into the biological processes underlying stress responses in corals and evolutionary adaptation to global climate change.

Page 1 of 18

IDT Exhibit 1006.0001 (page number not for citation purposes)



EX1006.0004 (Figure 2(b))

IPR2025-00015: Paper 41, 42-43; Paper 53, 18; IPR2025-00016: Paper 39, 38-39; Paper 51, 18; see also EX2038.¶¶260-265

Denominator Estimate: Based on the Average Transcript Length for Mammals

Dr. Meyer:

58. Second, we assumed that the average transcript size was 2,200 nucleotides. *Id.*, .0009. I believe this number was based on the average mRNA length in mammals, which had been reported to be 2,200 nucleotides. EX2080.

EX2287.¶58

Dr. Meyer Never Recanted His Testimony that the Average Transcript Length Is Based on Mammals

Can you point me to where in this Exhibit 2200 is mentioned?

A. Sure. So the page numbers for the exhibit described this at page 13 of 28, and if you go to the bottom of table 23.1, you will see that the average mRNA length for mammals is recorded at 2.2 kb, which is would be 2200 base pairs.

EX1126.0060:20-61:5 (Meyer Tr.)

Q. So if you had not received Dr. Matz's declaration that said it came from this textbook, you never would have gotten to this textbook, right?

A. I could only speculate about something like that. If I had not received it but had been requested to find a reference myself, I may or may not have identified the same reference. It was a part of general knowledge, and so that number likely appears in many different resources.

EX1126.0061:12-62:1

Denominator Estimate: Dr. Sims Suggests It Was Empirically Derived from Gel Reads

BMC Genomics

Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFLX

Elisavinda A. Gomes, Carlos V. Aguiar, Shi Wang, Jade Buchanan-Carter, David Abrego, John H. Colbourne, Bettina L. Willis and Mikhail V. Matz

phoresis to visualize the effectiveness of the process. A range of different sonication durations were tested, and the 3 minute treatment selected because it produced fragments in the appropriate size range (ca. 300–400 bp).

* * *

The cDNA is then:

* * *

- nebulized or sonicated to an average fragment size of 350-400 bp

EX1006.0013, 19

POSA's Expectation:

$$\frac{300 \text{ bp average fragment length}}{2,200 \text{ bp average transcript length}} \times 100\% \approx 14\%$$

$$\frac{350 \text{ bp average fragment length}}{2,200 \text{ bp average transcript length}} \times 100\% \approx 16\%$$

$$\frac{400 \text{ bp average fragment length}}{2,200 \text{ bp average transcript length}} \times 100\% \approx 18\%$$

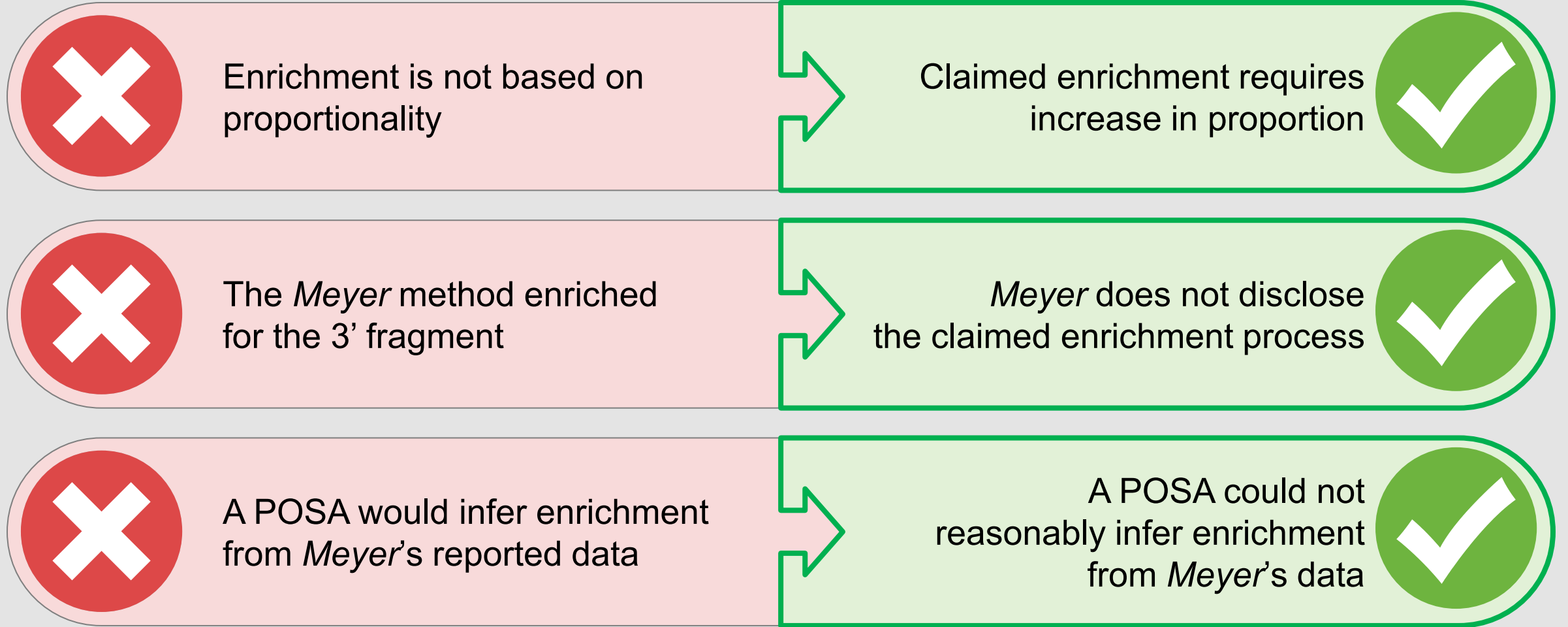
Dr. Meyer Confirms Lack of Data to Infer Enrichment

Certainly, we produced a sequence dataset that included 5' ends of genes, 3' ends of genes, and internal regions of genes. We produced dataset that wasn't completely lacking in any one of those three categories.

I don't -- I don't think we have the data at hand to evaluate whether -- whether the frequency of those fragments in the final pool was more or less than it started at or more or less than the other fragments. But I can say in terms of presence and absence, we achieved the goal in term of representing all of the regions of the -- of the genes.

EX1126.0179:18-180:10 (Meyer Tr.)

The Petition Suffers Three Fatal Flaws



Key Points

➤ **Meyer** does not anticipate claim 1 of the '012 and '108 patents

- Challenged claims require enrichment
- Meyer does not disclose enrichment of any sequences targeted by the target-specific adaptors

➤ **Meyer** does not anticipate claim 21 of the '012 patent

- Claim 21 requires that the claimed nucleic acid fragment be obtained by reverse transcribing an RNA fragment
- Meyer does not disclose fragmentation of RNA prior to reverse transcription

➤ **Meyer** does not anticipate claim 22 of the '012 patent

- Meyer does not disclose adaptors ligated to both ends of Petitioner's target nucleic acid fragments

➤ Petitioner has failed to show that any challenged claims are obvious

Claim 21

1. A method for sequencing an enriched nucleic acid sequence of interest, the method comprising:

* * *

21. The method of claim 1, wherein the nucleic acid fragment of the nucleic acid fragment ligated to the partial duplex adaptor in step a) is obtained by performing reverse transcription on an RNA fragment prior to obtaining the nucleic acid fragment ligated to the partial duplex adaptor in step a).

EX1001

“RNA” and “RNA fragments” Are Distinct

Term	Tecan’s Construction	Petitioner’s Construction
“RNA fragment”	“an RNA polynucleotide product generated through fragmentation”	“any RNA polynucleotide”

Tecan's Proposed Construction Is Supported by the Record

16. The method of claim 1, further comprising fragmenting a nucleic acid, thereby generating a nucleic acid fragment; and purifying the nucleic acid fragment prior to obtaining the nucleic acid fragment ligated to the partial duplex adaptor in step a).

* * *

20. The method of claim 1, further comprising fragmenting a nucleic acid, thereby generating a nucleic acid fragment; and performing end repair on the nucleic acid fragment prior to obtaining the nucleic acid fragment ligated to the partial duplex adaptor in step a).

EX1001

acid sequences from a sample comprising nucleic acids. In one aspect, the method comprises fragmenting nucleic acids in an input sample to generate nucleic acid fragments. The

* * *

In one embodiment, the input can be RNA. In one embodiment, the RNA can be obtained and purified using standard techniques in the art and include RNAs in purified or unpurified form, which include, but are not limited to, mRNAs, tRNAs, snRNAs, rRNAs, retroviruses, small non-coding RNAs, microRNAs, polysomal RNAs, pre-mRNAs, intronic RNA, viral RNA, cell free RNA and fragments thereof. The non-coding RNA, or ncRNA may include

EX1001 (6:51-53, 19:58-65)

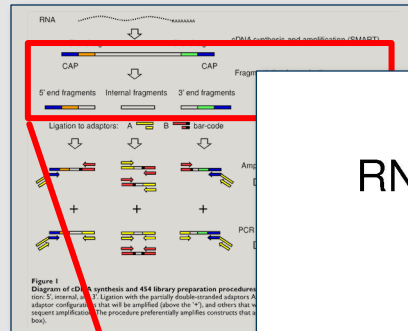
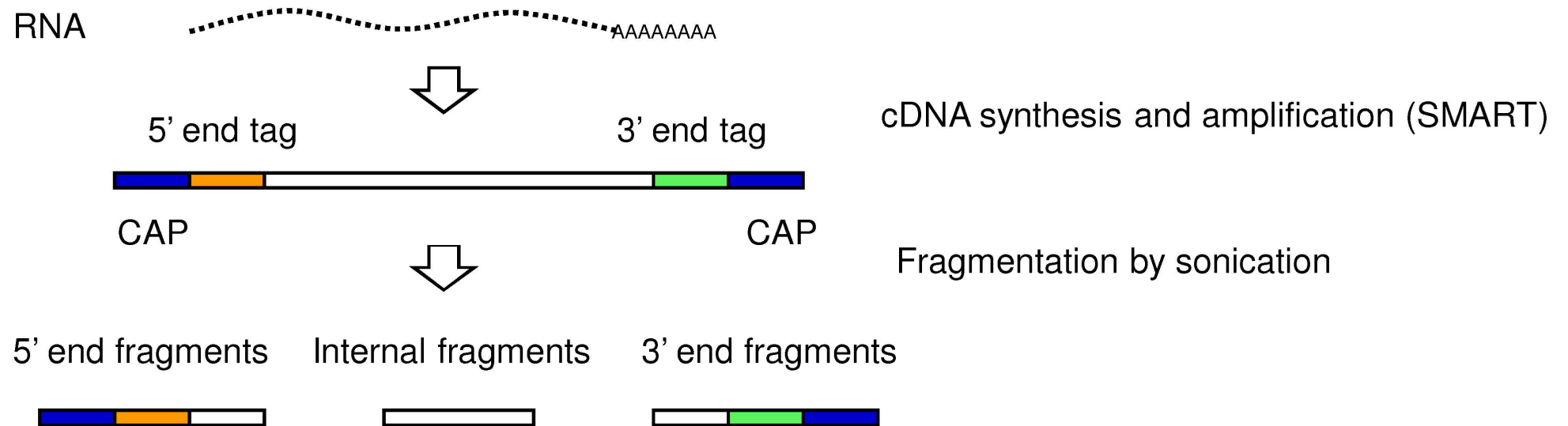
Tecan's Proposed Construction Is Supported by the Record



[I]n a case such as this, where the patent describes multiple embodiments, every claim does not need to cover every embodiment.

Pacing Techs., LLC v. Garmin Int'l, Inc.,
778 F.3d 1021, 1026 (Fed. Cir. 2015)

***Meyer* Fails to Disclose the Claimed Reverse Transcription of an RNA Fragment**



EX1006.0003

Key Points

➤ **Meyer** does not anticipate claim 1 of the '012 and '108 patents

- Challenged claims require enrichment
- Meyer does not disclose enrichment of any sequences targeted by the target-specific adaptors

➤ **Meyer** does not anticipate claim 21 of the '012 patent

- Claim 21 requires that the claimed nucleic acid fragment be obtained by reverse transcribing an RNA fragment
- Meyer does not disclose fragmentation of RNA prior to reverse transcription

➤ **Meyer** does not anticipate claim 22 of the '012 patent

- Meyer does not disclose adaptors ligated to both ends of Petitioner's target nucleic acid fragments

➤ Petitioner has failed to show that any challenged claims are obvious

Claim 22

1. A method for sequencing an enriched nucleic acid sequence of interest, the method comprising:

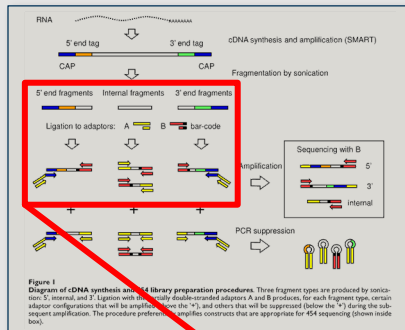
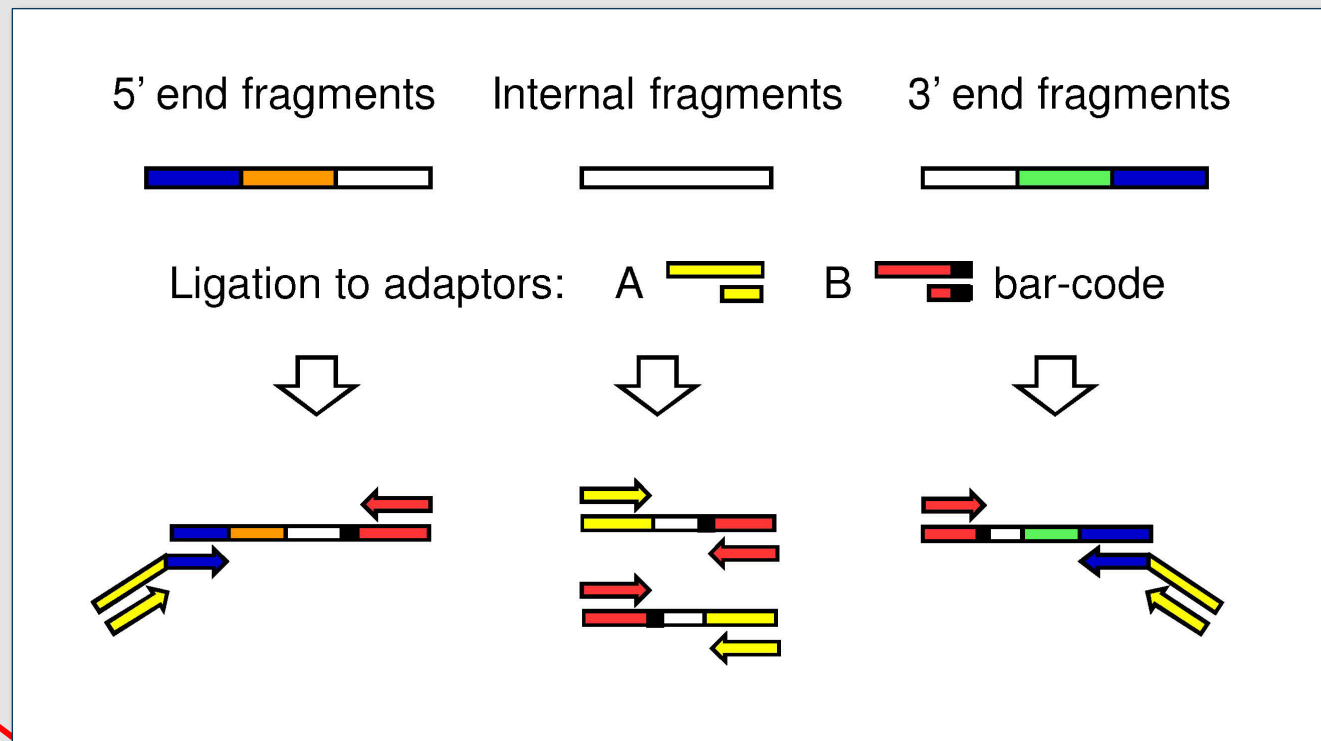
- a) obtaining a nucleic acid fragment ligated to a partial duplex adaptor, wherein the nucleic acid fragment comprises a nucleic acid sequence of interest, wherein

* * *

22. The method of claim 1, wherein each end of the nucleic acid fragment is ligated to a partial duplex adaptor.

EX1001

Meyer Does Not Disclose the Claimed Ligation on Both Ends of the Target Fragments



EX1006.0003

It is important to note that the adaptors only get ligated to the “new” 5’ ends formed as a result of fragmentation/polishing, since the original 5’ termini correspond to the incorporated “cap” primer used for amplification and lack 5’ phosphates.

Petitioner Must Show Inherent Anticipation



Moreover, a prior art reference may anticipate without disclosing a feature of the claimed invention if that missing characteristic is necessarily present, or inherent, in the single anticipating reference.

Schering Corp. v. Geneva Pharms., 339 F.3d 1373, 1377 (Fed. Cir. 2003)



In the context of accidental anticipation, [the claimed compound] DCL is not formed accidentally or under unusual conditions when loratadine is ingested. The record shows that DCL necessarily and inevitably forms from loratadine under normal conditions. DCL is a necessary consequence of administering loratadine to patients.

Schering Corp. v. Geneva Pharms., 339 F.3d 1373, 1378 (Fed. Cir. 2003)

Dr. Sims Concedes That There Is No Express Disclosure of the Method of Claim 22

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTEGRATED DNA TECHNOLOGIES, INC.,
Petitioner,

v.

TECAN GENOMICS, INC.,
Patent Owner.

IPR2025-00015
IPR2025-00016

U.S. Patents 10,036,012 and 10,876,108

DECLARATION OF PETER A. SIMS, PH.D.

IDT Exhibit 1085.0001

EX1085.¶263

IPR2025-00015: Paper 53, 22-23

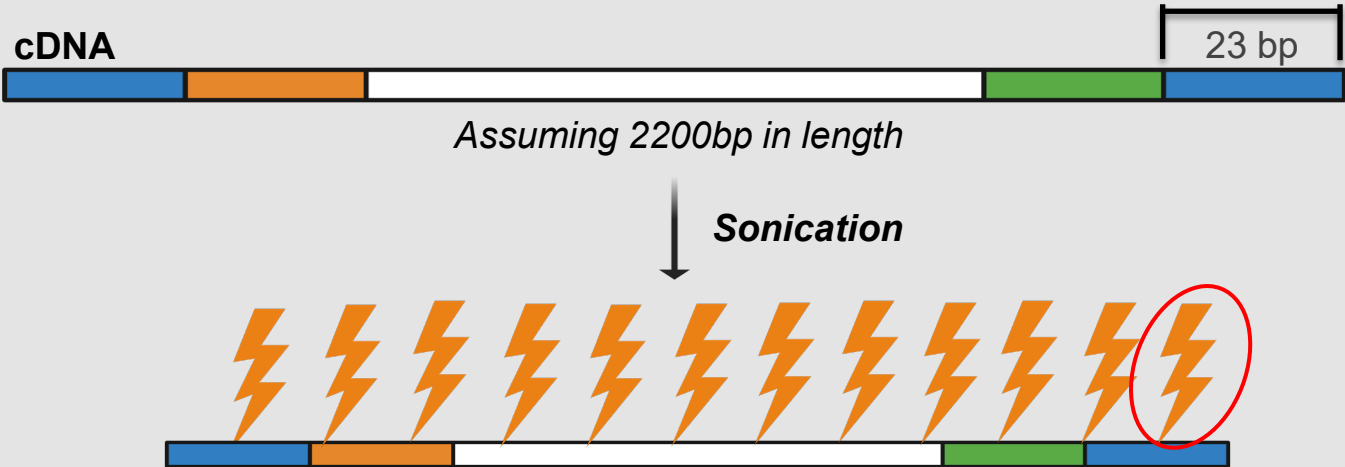
Demonstrative Exhibit - Not Evidence

263. The existence of these fragments is consistent with the POSA's understanding of the teachings of Meyer that some 3' end fragments were sheared in the cap region during sonication (and thus went through the other steps of the method outlined above). These observed sequences *are not* the fragments that actually

underwent all of the steps of the method because in those fragments the blue cap and red B adaptor would have been replaced by the A adaptor primer tail of the A+TRSAC primer during the anchored PCR reaction. Because of this replacement

of the adaptor with the primer tail, the fragments that underwent all the steps were at the end of the process indistinguishable from other fragments that were not ligated to adaptors on both ends (and in any event also would have been read *from the other end* from the red adaptor). But the observed sequencing reads I show above confirm the POSA's understanding that sonication created 3' fragments that were ligated to adaptors on both ends as evidenced by the fact that hundreds of reads showed adaptors on both ends of fragments containing the green broken chain polyT adaptor sequence and the blue cap sequence.

Petitioner Fails to Show that Fragmentation Will Necessarily and Inevitably Damage Any Cap Primers in All Instances



Key Points

➤ **Meyer** does not anticipate claim 1 of the '012 and '108 patents

- Challenged claims require enrichment
- Meyer does not disclose enrichment of any sequences targeted by the target-specific adaptors

➤ **Meyer** does not anticipate claim 21 of the '012 patent

- Claim 21 requires that the claimed nucleic acid fragment be obtained by reverse transcribing an RNA fragment
- Meyer does not disclose fragmentation of RNA prior to reverse transcription

➤ **Meyer** does not anticipate claim 22 of the '012 patent

- Meyer does not disclose adaptors ligated to both ends of Petitioner's target nucleic acid fragments

➤ **Petitioner has failed to show that any challenged claims are obvious**

Roadmap

Ground 2 (all challenged claims of '012; claims 8, 17, 18 of '108):

Petitioner's Claims that *Meyer* Alone Renders Obvious Patent Claims Is Legally Insufficient

Ground 3 (claim 2 of both): Petitioner Fails to Show How a POSA Would Modify *Meyer* Based on Siebert to Practice the Challenged Claims and Why a POSA Would Be Motivated to Do So With a Reasonable Expectation of Success

Ground 4 (claim 9 of '012): Fails for the Same Reasons as Grounds 1 and 2

Ground 5 (claims 15, 17-19 of '012; claims 11, 12, 19 of '108):

Petitioner Relies on References that Teach Away and Fails to Show Why a POSA Would Be Motivated to Modify *Meyer* for Use With Other NGS Systems

Secondary Considerations:

1. Prior Art Taught Away and Expressed Skepticism;
2. Long-Felt, Unmet Needs;
3. Industry Praise

Ground 2 Is Legally Insufficient

Petition for IPR2025-00015

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTEGRATED DNA TECHNOLOGIES, INC.,
Petitioner,

v.

TECAN GENOMICS, INC.,
Patent Owner.

Case IPR2025-00015
U.S. Patent 10,036,012

**PETITION FOR *INTER PARTES* REVIEW
OF U.S. PATENT 10,036,012**

**Ground 2: In The Alternative, Claims 1, 3, 5-8, 10-14, 16, And 20-22
Would Have Been Obvious Over Meyer.**

Paper 1, 67

Petition for IPR2025-00016

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTEGRATED DNA TECHNOLOGIES, INC.,
Petitioner,

v.

TECAN GENOMICS, INC.,
Patent Owner.

Case IPR2025-00016
U.S. Patent 10,876,108

**PETITION FOR *INTER PARTES* REVIEW
OF U.S. PATENT 10,876,108**

**Ground 2: In The Alternative, Claims 8, 17, And 18 Would Have Been
Obvious Over Meyer.**

Paper 1, 61

IPR2025-00015: Paper 41, 48-50; IPR2025-00016: Paper 39, 41-43

Claim 2

10,036,012

1. A method for sequencing an enriched nucleic acid sequence of interest, the method comprising:

* * *

2. The method of claim 1, wherein the nucleic acid fragment comprises genomic DNA.

EX1001

10,876,108

1. A method for sequencing an enriched nucleic acid sequence of interest, the method comprising:

* * *

2. The method of claim 1, wherein the nucleic acid sequence of interest comprises genomic DNA.

EX1002

Ground 3: Siebert

An improved PCR method for walking in uncloned genomic DNA

Paul D. Siebert*, Alex Chenchik, David E. Kellogg, Konstantin A. Lukyanov¹ and Sergey A. Lukyanov¹

Clontech Laboratories, Inc., 4030 Fabian Way, Palo Alto, CA 94303-4607, USA and ¹Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaja 16/10, V-437 Moscow 117871, Russia

Received January 4, 1995; Accepted February 9, 1995

Several PCR-based methods are available for walking from a known region to an unknown region in cloned or uncloned genomic DNA. The methods are of three types: inverse PCR (1), randomly primed PCR (2) and adaptor ligation PCR (3-6). However, these methods have not been generally applied to walking in uncloned genomic DNA because they are either complicated or inefficient. Recent improvements to these methods have been applied to uncloned genomic DNA (7,8), however, walks have been limited to distances of <1 kb.

We have investigated the application of 'long and accurate PCR' (9,10) to walking in uncloned genomic DNA. We initially examined the use of 'unpredictably primed PCR' (3), a new method based on randomly primed PCR and 'vectorette PCR' (5), which is based upon adaptor ligation. As a model system we attempted to walk upstream from exon 1 of the human tissue-type plasminogen activator (TPA) gene which has been previously characterized (11).

In our hands both 'unpredictably primed PCR' and 'vectorette PCR' generated multiple PCR products even after nested PCR was performed. Although upon analysis some of the PCR products were found to be derived from successful walks, the presence of multiple PCR products would complicate further characterization without Southern blot hybridization or extensive cloning.

We have improved upon the adaptor ligation method by combining 'vectorette PCR' with a newly developed method termed 'suppression PCR' (12). A special adaptor is ligated to the ends of DNA fragments generated by digestion of human genomic DNA with *EcoRV*, *ScaI*, *DraI*, *PvuII* and *SspI* separately. Following adaptor ligation, a small amount of the DNA is used as a template for PCR using adaptor primers and gene specific primers. The enzymes used were selected because they have six-base recognition sites and generate blunt ends.

The sequences of the walking adaptor and primers are shown in Figure 1. One end of the adaptor is blunt so that it will ligate to both ends of any DNA fragment generated by restriction enzymes that yield blunt ends. The adaptor also contains two rare restriction enzyme sites for *NcoI* (staggered ends) and *SrfI*/*SmaI* (blunt ends) to allow cloning into commonly used vectors such as pBluescript (Stratagene).

The 'vectorette' feature of the adaptor is the presence of an amine group on the 3'-end of the lower strand. This blocks

Nucleic Acids Research, 1995, Vol. 23, No. 6 1087-1088

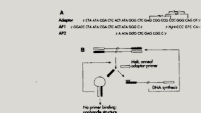


Figure 1. (A) Sequences of the walking adaptor and adaptor primers and gene-specific primers. (B) Illustration of the suppression PCR effect. Structure of PCR products formed by non-specific amplification. The sequences on the ends of the products contain inverted terminal repeats and will form 'panhandle' structures which suppress PCR.

polymerase catalyzed extension of the lower adaptor strand, preventing the generation of the primer binding site unless a defined, distal, gene-specific primer extends a DNA strand opposite the upper strand of the adaptor. The 'suppression PCR' technology uses an adaptor primer which is shorter in length than the adaptor and is capable of hybridizing to the outer primer binding site. If any PCR products are generated which contain double-stranded adaptor sequences at both ends (due to non-specific DNA synthesis), the ends of the individual DNA strands will form 'panhandle' structures following every denaturation step, due to the presence of inverted terminal repeats (Fig. 1B). These structures are more stable than the primer-template hybrid and therefore will suppress exponential amplification. However, when a distal gene-specific primer extends a DNA strand through the adaptor, the extension product will contain the adaptor sequence only on one end and thus cannot form the 'panhandle' structure. PCR amplification can then proceed normally.

To prepare the adaptor-ligated DNA, 2.5 µg of human genomic DNA (Clontech) is digested in 100 µl reaction volumes with 80 U of restriction enzyme overnight at 37°C using buffers recommended by the supplier (Life Technologies). The DNA was extracted once with phenol/chloroform/isoamyl alcohol (25:24:1) vol/vol, once with chloroform, and then precipitated by addition of 1/10th vol 3 M NaOAc, 20 µg glycogen (molecular biology grade, Boehringer Mannheim) and 2 vol of 95% EtOH.

* To whom correspondence should be addressed

IDT Exhibit 1007.0001

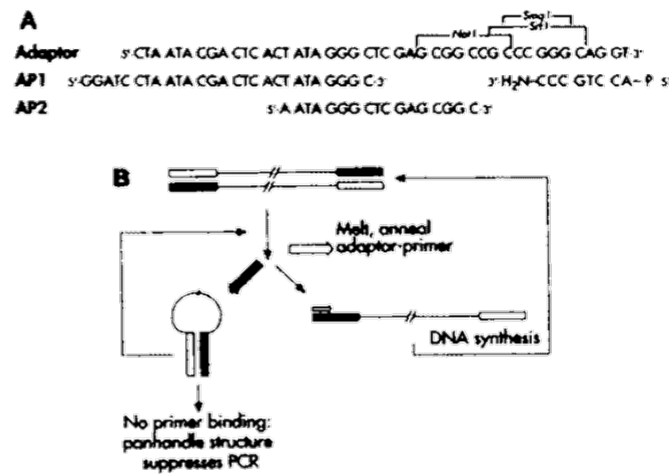


Figure 1. (A) Sequences of the walking adaptor and adaptor primers and gene-specific primers. **(B)** Illustration of the suppression PCR effect. Structure of PCR products formed by non-specific amplification. The sequences on the ends of the products contain inverted terminal repeats and will form 'panhandle' structures which suppress PCR.

EX1007.0001

Ground 3: A POSA Would Not Be Motivated to Combine *Meyer* With Siebert to Practice the Claimed Invention

- 1 A POSA would be discouraged from using *Meyer* with genomic DNA.
- 2 Siebert and *Meyer* are addressing different problems.
- 3 *Meyer* sought to solve a specific sequencing issue with the 454 sequencer.
- 4 The prior art teaches away from the claimed amplification with single-target-specific primers.

Ground 3: *Meyer's* Reliance on the SMART Protocol Would Discourage Adaptation With Genomic DNA

SMART Protocol (EX1034.0005)

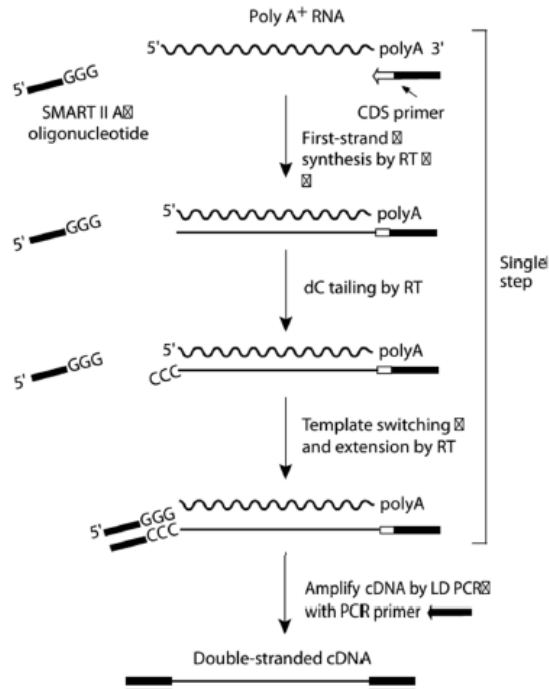
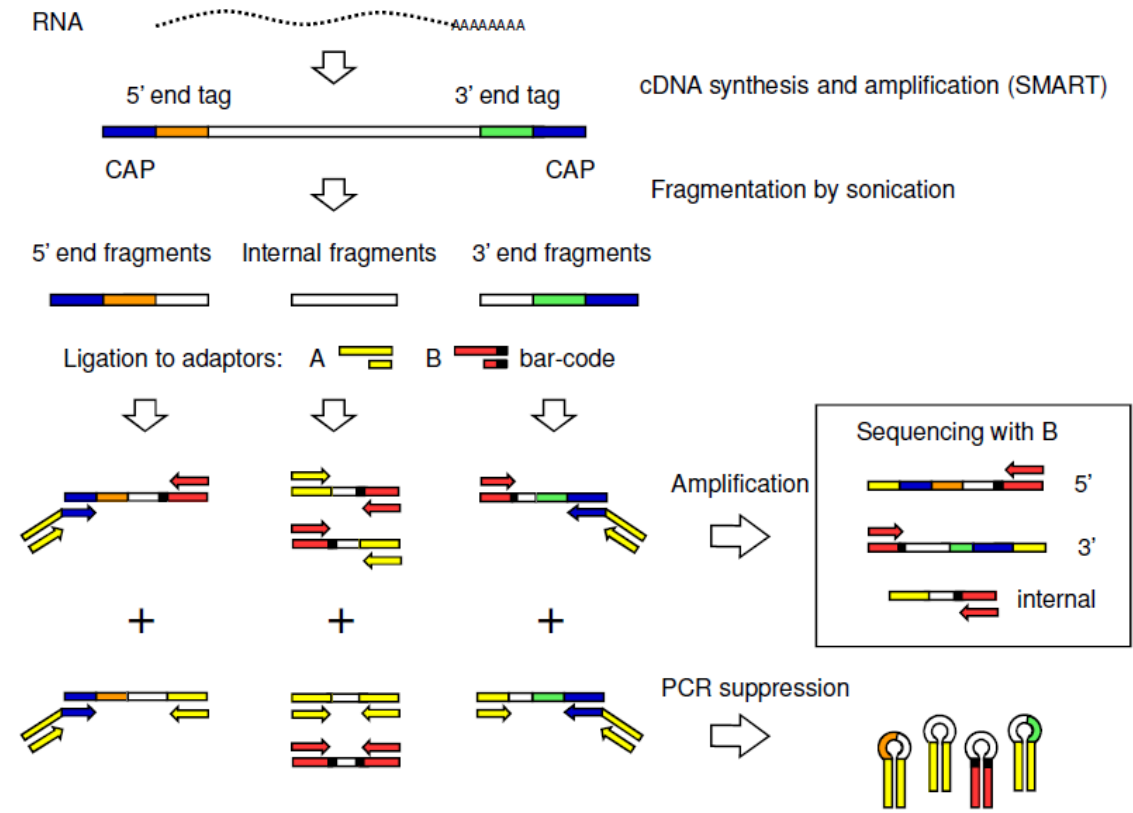


Figure 1. Flow chart of SMART™ technology. The SMART II A Oligonucleotide, 3' SMART CDS Primer II A, and 5' PCR Primer II A all contain a stretch of identical sequence (see Section II for complete sequence information).

Meyer Protocol (EX1006.0003)



Ground 3: Siebert Would Have Discouraged the Claimed Method

Siebert

An improved PCR method for walking in uncloned genomic DNA

Paul D. Siebert*, Alex Chenchik, David E. Kellogg, Konstantin A. Lukyanov¹ and Sergey A. Lukyanov¹

Clontech Laboratories, Inc., 4030 Fabian Way, Palo Alto, CA 94303-4607, USA and ¹Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaja 16/10, V-437 Moscow 117871, Russia

Received January 4, 1995; Accepted February 9, 1995

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The sequences of the walking adaptor and primers are shown in Figure 1. One end of the adaptor is blunt so that it will ligate to both ends of any DNA fragment generated by restriction enzymes that yield blunt ends. The adaptor also contains two rare restriction enzyme sites for NotI (staggered ends) and SrfI/SmaI (blunt ends) to allow cloning into commonly used vectors such as pBluescript (Stratagene).

The 'vectorette' feature of the adaptor is the presence of an amine group on the 3'-end of the lower strand. This blocks

Nucleic Acids Research, 1995, Vol. 23, No. 6 1087-1088



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polymerase catalyzed extension of the lower adaptor strand, preventing the generation of the primer binding site unless a defined, distal, gene-specific primer extends a DNA strand opposite the upper strand of the adaptor. The 'suppression PCR' technology uses an adaptor primer which is shorter in length than the adaptor and is capable of hybridizing to the outer primer binding site. If any PCR products are generated which contain double-stranded adaptor sequences at both ends (due to non-specific DNA synthesis), the ends of the individual DNA strands will form 'panhandle' structures following every denaturation step, due to the presence of inverted terminal repeats (Fig. 1B). These structures are more stable than the primer-template hybrid and therefore will suppress exponential amplification. However, when a distal gene-specific primer extends a DNA strand through the adaptor, the extension product will contain the adaptor sequence only on one end and thus cannot form the 'panhandle' structure. PCR amplification can then proceed normally.

To prepare the adaptor-ligated DNA, 2.5 µg of human genomic DNA (Clontech) is digested in 100 µl reaction volumes with 80 U of restriction enzyme overnight at 37°C using buffers recommended by the supplier (Life Technologies). The DNA was extracted once with phenol/chloroform/isoamyl alcohol (25:24:1) vol/vol, once with chloroform, and then precipitated by addition of 1/10th vol 3 M NaOAc, 20 µg glycogen (molecular biology grade, Boehringer Mannheim) and 2 vol of 95% EtOH.

* To whom correspondence should be addressed

IDT Exhibit 1007.0001

genomic DNA. The methods are of three types: inverse PCR (1), randomly primed PCR (2) and adaptor ligation PCR (3-6). However, these methods have not been generally applied to walking in uncloned genomic DNA because they are either complicated or inefficient. Recent improvements to these

* * *

In our hands both 'unpredictably primed PCR' and 'vectorette PCR' generated multiple PCR products even after nested PCR was performed. Although upon analysis some of the PCR products were found to be derived from successful walks, the presence of multiple PCR products would complicate further characterization without Southern blot hybridization or extensive cloning.

* * *

We have found that the high specificity of the method described is primarily due to the suppression effect of the adaptor/adaptor primer combination, although in some cases additionally having an amine group on the lower strand of the adaptor (vectorette blocking) yielded better results.

EX1007.0001-02

Ground 3: Other Genome Walking Papers Discouraged the Claimed Method

Miura (2005)

competitive PCR (ATAC-PCR) (Kato, 1997) and so forth. The specificity of the PCR in these applications depends solely on that of the gene-specific primer. Accordingly, they are often plagued with non-specific amplification products derived from so-called ‘off-targets.’

Abstract: These applications include rapid amplification of cDNA ends (RACE), adaptor-tagged competitive PCR (ATAC-PCR), PCR-mediated genome walking and so forth. Since the primers designed by conventional methods often fail to work in these applications, an alternative strategy is required, particularly for a large-scale analysis. **Results:** Based on the structure of off-target products in the ATAC-PCR, we reasoned that the practical determination of the specificity of primers may not be the uniqueness of entire sequence but that of the sequence of the primer itself, that is, the sequence of the 3' flanking region. We termed such a subsequence as a 'specificity-determining subsequence' (SDSS) and developed a simple algorithm to predict the performance of the primer; the algorithm identifies the SDSS of the primer and the sequence of the 3' flanking region of the template. The primers designed using this algorithm worked much better than those designed using a conventional method in both ATAC-PCR and 5'-RACE experiments. Thus, the algorithm will be generally useful for improving the efficiency of primer design. **Conclusion:** Supplementary data are available at http://www.biomedcentral.com/info/publishing_adv.asp. **Availability:** The source code of the program is available upon request for non-commercial use or can be obtained from <http://tobaib.cb.k-u-tokyo.ac.jp/GATC/>. **Supplementary Information:** Supplementary data are available at http://www.biomedcentral.com/info/publishing_adv.asp. **Keywords:** 5'-RACE, ATAC-PCR, primer design, algorithm

1 INTRODUCTION

Conventional PCR uses a pair of unique primers to selectively amplify a target sequence. The specificity of amplification is thus achieved by the synergistic effect between the specificities of the two primers. In contrast, some PCR applications use only a single unique primer in combination with a common primer. These include rapid amplification of cDNA ends (RACE) (Frohman *et al.*, 1988), PCR-mediated genome walking (Shyamala and Ames, 1989; Riley *et al.*, 1990), adaptor-tagged competitive PCR (ATAC-PCR) (Kato, 1997) and so forth. The specificity of the PCR in these applications depends solely on

*To whom correspondence should be addressed.

including the annealing temperature and the concentrations of Mg^{2+} , dNTPs, primers and the template. While methods have been developed for the efficient optimization of PCR conditions (Cobb and Clarkson, 1994), it is impractical to perform such adjustments on each of the thousands of primers used in a large-scale experiment. The most reliable solution that would require no preliminary experimentation is the use of a general primer set. In addition to the general primer set, specific primers (Gibbons et al., 1991). However, this would inevitably double the cost and time. Thus, a reliable method is required to design more specific primers, particularly, for use in a large-scale analysis based on these PCR applications. In addition, the primers should work efficiently under a broad range of experimental conditions so that a single unique condition can be used throughout the large-scale experiment. Such primers would be useful if it is possible to optimize the PCR conditions for a single primer set and then use the same PCR conditions for all the primers.

We are developing a genome-wide ATAC-PCR system for the budding yeast *Saccharomyces cerevisiae*. For these experiments, we had designed each gene-specific primer such that the 12mer including its 3'-end (3'-end 12mer) is unique in the yeast genome. Even with these primers, we often encountered off-target products. To determine the etiology of these off-target products, they were cloned and sequenced. As was expected, we found that they were generated by the misannealing of gene-specific primers and, more importantly, that the length of the homologous sequence shared by the primer and the off-target differed from one case to another.

Based on these observations, we assumed that, at least in some instances, the practical specificity may be better determined by the uniqueness of the minimal 3'-end subsequence that exceeds the threshold of duplex stability rather than the uniqueness of entire primer or that of a 3'-end subsequence with a fixed length. We termed such a subsequence as a 'specificity-determining subsequence' (SDSS). Increasing the uniqueness of the SDSS is generally equivalent to its elongation. The longer the SDSS, the more likely it is that the primer functions as a unique one. The shorter the SDSS, the more likely it is that the primer would find off-targets. Primers with a longer SDSS tend to be more *AT* rich in their 3'-end portions, and the duplex formed by this region is rather unstable.

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EX2047.00001

Tsoktouridis (2005)

Several PCR methods have been described to amplify unknown DNA fragments using specific DNA

* * *

Comparison of these methods revealed no simplicity, low reproducibility, high backgrounds of nonspecific PCR products, inefficient homopolymer tailing, small PCR product amplification, and laborious time-consuming procedures (5–8,11,12).

EX2048.0002

DNA fragments using specific DNA polymerases (3,4), different types

in large unknown DNA sequences adjacent to a particular

SHORT TECHNICAL REPORT

e for clone-
mosomal

elVecchio

developed to detect genomic
ly related strains of bacte-
high-speed experimental
y, inversions, duplications,

- advantages of this strategy
- do not use reaction mixtures

6-min ligation procedure; site concentration protocol; wide-range PCR protocol to cycling with the same adaptors according to technology and equipment; reverse subtractive hybridization; deletion and a 7255-bp cella abortus 2308 used as

tide primers such as oligonucleotide-primed R; Reference 5), linker ed PCR (LAM-PCR; rapid amplification of ACE-PCR; Reference 6), competitive PCR (Reference 7), serial expression (SAGE; Reference 8), and long and accurate R; Reference 10), these methods revealed low reproducibility, ds of nonspecific PCR efficient homopolymer PCR product amplification time-consuming

Generation of DNA Adaptor-Linked Digests

ture 1) to examine each *Brucella melitensis* tester and Brucella as the driver (13). The 2.308 will be used to species throughout this specifically, ALR-PCR to identify genomic differences in large unknown sequences adjacent to a particular

The cohesive-ended restriction endonuclease *SphI* (New England Biolabs, Beverly, MA, USA) was used to digest 2308 genomic DNA in a reaction mixture of 50 μ l containing 5 μ l of 10x restriction endonuclease enzyme buffer (NEB 2; New England Biolabs), 1100 ng DNA, and 5 U restriction endonuclease enzyme for 4 h at 37°C and then purified using

BioTechniques 885

Tecan Ex. 2048, Page 2 of 5
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IPR2025-00015: Paper 41, 52-53, 60-62; Paper 53, 23-24; IPR2025-00016: Paper 39, 45-46, 52-54; Paper 51, 21-22; see also EX2038. ¶¶ 62-67, 315-317, 348-350

Demonstrative Exhibit - Not Evidence

Ground 3: Other Genome Walking Papers Would Have Discouraged the Claimed Method

Sohn

The most serious problems of the PCR-based genome walking methods are low amplification efficiency and non-specific amplification. That is, unwanted genes are amplified together, which makes it difficult to distinguish amplification of desired genes. To improve this problem, complicated multi-step methods were developed, including secondary PCR, purification of desired PCR products using biotinylated primers, or improvement of PCR cycle conditions. However, satisfactory performance has not yet been achieved.

EX2050.0003

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(54) Title: A NOVEL GENOME WALKING METHOD FOR CLONING OF UNKNOWN DNA SEQUENCES
KNOWN SEQUENCE

(Fig. 2)

(57) Abstract: The present invention relates to a novel genome walking method for cloning unknown sequences by PCR. More particularly, the present invention relates to a method for cloning a sequence adjacent to a known nucleotide sequence, comprising the steps of 1) cleaving a genomic DNA with a restriction enzyme; 2) linking the terminal sequence of the cleaved DNA with a nucleotide sequence complementary thereto; and 3) amplifying the unknown nucleotide sequence using a known nucleotide sequence-specific primer and a cassette sequence-specific primer. Further, the present invention relates to a composition of gene amplification for cloning the unknown nucleotide sequence, and a gene amplification kit comprising the composition.

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IPR2025-00015: Paper 41, 52-53, 60-62; Paper 53, 23-24; IPR2025-00016: Paper 39, 45-46, 52-54; Paper 51, 21-22;
see also EX2038. ¶¶62-67, 315-317, 348-350

Ground 3: Leoni

Leoni

FEBS
Journal

REVIEW ARTICLE

Genome walking in eukaryotes

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Keywords

gene trapping; gene therapy; gene trapping; genome walking; insertional mutagenesis; multigene family; next generation sequencing; T-DNA; transposable elements; virus integration

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Introduction

Identification of unknown nucleotide sequences flanking already characterized DNA regions can be pursued by a number of different PCR-based methods commonly known as genome walking (GW).
In times of high-throughput DNA sequencing technologies, when more than 1000 genomes have been completely sequenced, the development of GW strategies can appear as an out-of-date laboratory activity. Nevertheless, papers describing applications of GW methods and improvements of several available strate-

gies continue to be published with a steady positive trend. Reasons for such constant interest can be found both in the relatively low difficulty of the different strategies, which do not require expensive equipment or highly trained personnel, and in the increasing possibilities of applying GW methods to eukaryotic genomes. Furthermore, in some highly sophisticated applications, GW strategies have recently been combined with pyrosequencing technology allowing the production of hundreds of thousands of sequences per

Abbreviations

ASLV, avian sarcoma-leukosis virus; blocked DLA, blocked d'ligation-ampification; EC-PCR, extension and cassette PCR; E-GW, extension-based GW; E1-PCR, extension and ligation PCR; FLEA-PCR, flanking sequence exponential anchored PCR; GW, genome walking; I-PCR, inverted-PCR; LAM-PCR, linear amplification on mediated PCR; LM-PCR, ligation mediated PCR; MLV, murine leukemia virus; P-GW, primer-based GW; RAGE, rapid amplification of genomic ends; R-GW, restriction-based GW; SHP-PCR, sequential hybrid primer PCR; TAIL-PCR, thermal asymmetric lobe-acid PCR; T-DNA, transfer DNA; TVL-PCR, TOPO² vector-ligation PCR; UVW, universal fast walking.

3953

FEBS Journal 278 (2011) 3953–3977 Journal compilation © 2011 FEBS. No claim to original Italian government works

IDT Exhibit 1052.0001

EX1052.0003 (Table 1)

Table 1. GW strategies. GW methods are catalogued as R-GW, P-GW and E-GW. When available, the specific name of the method as given by the authors is reported. Otherwise the name of the first author is given. Methods in gray boxes are detailed in the GW methods section. P and E columns refer to the analysis of prokaryotic and eukaryotic genomes, respectively.

	Group	Sub-group	Name	References	P	E
1	R-GW	Inverted PCR	Inverted PCR	5		×
2			Long range inverted PCR	6		×
3			Bridged inverted PCR	7	×	
4		Cassette PCR	Rolling circle inverted PCR	8		×
5			Single-specific-primer PCR	9	×	
6			Fors <i>et al.</i>	10		×
7			Vectorette PCR	11		×
8			Cassette ligation	12		×
9			Capture PCR	13		×
10			Splinkerette PCR	14		×
11			Boomerang DNA amplification	1		×
12			Suppression PCR GW	15		×
13	P-GW	Others	Padegimas and Reichert	16		×
14			Step-down PCR	17		×
15			Simplified oligo-cassette	18	×	
16			Cottage <i>et al.</i>	19		×
17			T-DNA fingerprinting PCR	20		×
18			T-linker PCR	21		×
19			Versatile cassette	22	×	
20			Barcoding pyrosequencing	23,24		×
21			One-base excess adaptor ligation	25		×
22			Straight walk	26		×
23			Blocked DLA	27		×
24			Template-blocking PCR	28	×	×
25			TVL-PCR	29		×
26			Panhandle PCR	30		×
27			Supported PCR	31		×
28			RAGE	32		×
29			Restriction site extension PCR	33		×
30		P-GW	Targeted gene-walking PCR	34		×
31			Restriction-site PCR	35		×
32			Restricted PCR (novel Aiu PCR)	36		×
33			TAIL-PCR	37		×
34			Uneven PCR	38		×
35			Semi-random PCR	39	×	
36			Mishra <i>et al.</i>	40		×
37			UFW PCR	41,42		×
38			Lariat-dependent nested PCR	43		×
39			Site finding PCR	44		×
40			Touchdown PCR-based	45	×	
41			Walser <i>et al.</i>	46		×
42	E-GW	EC-PCR	Nested PCR-based	47	×	
43			Self-formed adaptor PCR	48	×	
44			Two-step gene walking PCR	49	×	
45		ET-PCR	High-genome walking	50		×
46			SD-PCR	51	×	
47			SHP-PCR	52	×	
48		Others	LM-PCR	53		×
49			LAM-PCR	54		×
50			Long distance genome walking PCR	55		×
51			Leoni <i>et al.</i>	56,57		×
52			Single-primer amplification	58		×
53			FLEA-PCR	59		×

IPR2025-00015: Paper 53, 24; IPR2025-00016: Paper 51, 21-22

Demonstrative Exhibit - Not Evidence

55

Ground 5: *Meyer* Addresses Challenges Specific to the 454 Sequencer



Meyer

Discussion

Methodological advances for 454 sequencing of transcriptomes

The methods described here resolve several challenges associated with 454 sequencing of transcriptomes.

EX1006.0009

Secondary Considerations: Petitioner Misstates the Law on Nexus



If an applicant demonstrates that an embodiment has an unexpected result and *provides an adequate basis to support the conclusion that other embodiments falling within the claim will behave in the same manner*, this will generally establish that the evidence is commensurate with scope of the claims.

In re Huai-Hung Kao, 639 F.3d 1057, 1068 (Fed. Cir. 2011)
(highlighting Petitioner's relied-upon portion of the quote)

Secondary Considerations: Tecan Demonstrated Nexus

Long-Felt, Unmet Needs:



Given that the solution to the long-felt need results from the claimed and novel dosing regimens, nexus to the claimed invention is established.

Janssen Pharms., Inc. v. Teva Pharms. USA, Inc., 760 F. Supp. 3d 184, 217 (D.N.J. 2024), *aff'd*, 141 F.4th 1367 (Fed. Cir. 2025)

Industry Praise:



Thus, the praise relates to the “novel dose initiation procedure,” *id.* at 205, which establishes a nexus to the “claimed invention.”

Id. at 222

Skepticism:



Given that this skepticism was aimed directly at novel aspects of the claimed dosing regimen, it has a nexus to the patented invention.

Id. at 222

Secondary Considerations: The Prior Art Taught Away From Using PCR Amplification

Quail 2008

Sequencing without PCR. We have found that it is unnecessary to retain the PCR step to enrich for properly ligated fragments, so long as only those fragments with an adapter at either end can be quantified, as only they will yield clusters that can be sequenced. This can be done by quantitative PCR, discussed below. Thus we can eliminate the PCR step entirely, simply by ligating on appropriate adapters after A-tailing (Supplementary Protocol 9 online). For this purpose we use high-performance liquid chromatography-purified, partially noncomplementary oligos with a phosphorothioate linkage between the two bases at the 3' end of one strand. From a starting amount of 5 µg of DNA and fractionation by AFA, we can obtain sufficient paired-end DNA for > 400 lanes of high-density clusters, or 100 lanes if nebulization is used to fragment the DNA. The obvious benefits of this are that PCR duplicates are absent: the observed duplication

EX2053.0004

Quail 2011

Libraries prepared without PCR amplification¹ performed best. Among the amplified libraries there were big differences,

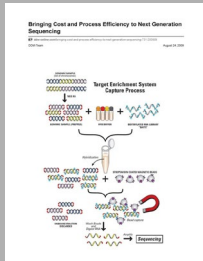
EX2071.0001

Kozarewa

G+C content. Here we present an amplification-free method of library preparation, in which the cluster amplification step, rather than the PCR, enriches for fully ligated template strands, reducing the incidence of duplicate sequences, improving read mapping and single nucleotide polymorphism calling and aiding *de novo* assembly. We illustrate this by generating and analyzing

EX2054.0001

Secondary Considerations: Long-Felt, Unmet Needs Persisted As of the Priority Date



Ernani,
EX2062.0003
(August 2009)

“While next-generation sequencing has revolutionised the way genomes are sequenced, this technology possesses **a fundamental weakness – the inability to easily target specific regions of a genome.**”



Moorthie,
EX1050.0007
(October 2011)

“**Key issues**, which apply to all methods with varying degrees are **uniformity, efficiency of coverage and off-target capture**. Whilst these methods are continually being improved, **it may ultimately be more cost-effective to sequence a whole genome**, computationally masking regions of the genome that are irrelevant to a particular clinical question, and target analysis only to regions with proven clinical significance.”



Grunenwald,
EX2056.0001
(August 2010)

“Current methods (both mechanical and enzymatic) face limitations: **multi-step protocols, sample loss, lack of automation and labor costs**. With the continued decline of sequencing costs and increase in sample throughput, there is **greater demand for more cost effective, streamlined library preparation methods.**”



Myllykangas,
EX2052.0002
(December 2011)

“Because the target enrichment and sequencing library preparation occur in succession, targeted resequencing methods **tend to be laborious, time-consuming, prone to experimental errors and difficult to automate**. In addition, many of these approaches have **complex protocols which require large amount of starting genomic DNA**. Other issues include being **susceptible to coverage biases secondary to amplification** and molecular artifacts.”

Secondary Considerations: Industry Praise

Barchi

Single primer enrichment technology (SPET) is a new, robust, and customizable solution for targeted genotyping. Unlike genotyping by sequencing (GBS), and like DNA chips,

Recently Nugen® developed the single primer enrichment technology (SPET, United States Patent 9,650,628), which is a customizable solution for targeted sequencing at an affordable price. SPET requires a priori genomic or transcriptomic

a primer. Its reliance on single primers, rather than on primer pairs, greatly simplifies panel design, and allows higher levels of multiplexing than PCR-based genotyping.

Single primer enrichment technology is a robust method, performing well with DNA samples prepared by different laboratories using different DNA mini-preparation protocols,

approaches (Scheben et al., 2017). Furthermore, SPET provides the ability of multiplexing thousands of samples in a single sequencing run, which can be genotyped with tens of thousands of probes, and with a good coverage at target sites. Finally, thanks to the sequencing of the genomic regions around the target SNPs, SPET allows the discovery of thousands of novel SNPs not originally included in the panel.

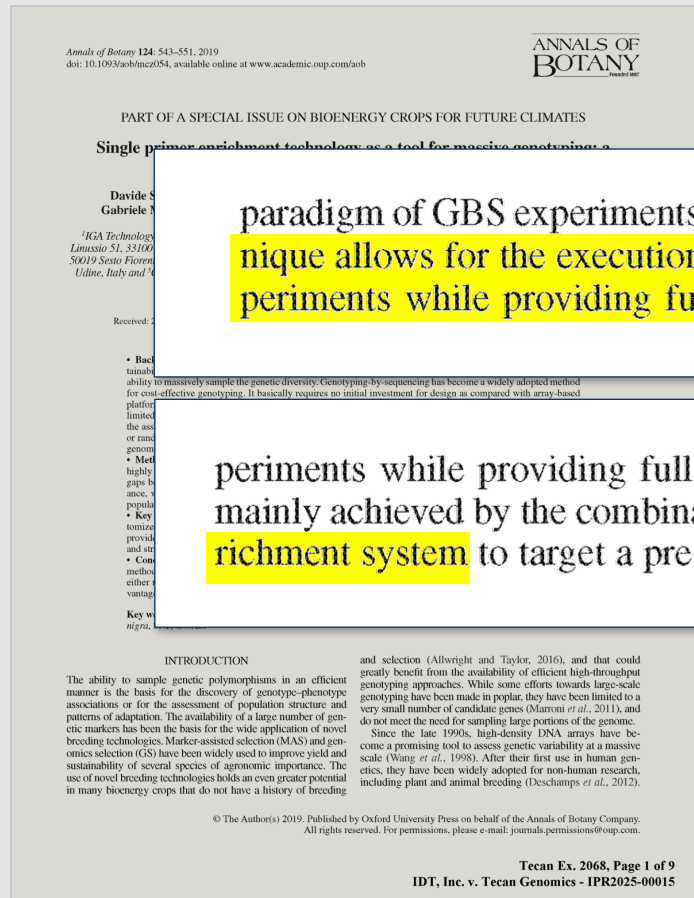
Secondary Considerations: Industry Praise

Smith

The SPET primers are targeted to both strands of the target sequence for more robust detection in SNP analysis, CNV analysis, and re-sequencing. Using single primers instead of primer pairs greatly simplifies the design of primer panels, and allows higher levels of multiplexing compared to amplicon-based enrichment.

EX2067.0003

Secondary Considerations: Industry Praise



Scaglione

given locus; (3) the scalability to tens of thousands of probes in a single reaction; and (4) the removal of PCR duplicates even in the absence of variation of the mapping co-ordinates for a

EX2068.0006

in every locus. Our results suggest that SPET genotyping is a very effective approach for genotyping a large number of loci, and we suggest that it might be a valuable tool for large-scale genotyping studies in virtually all species. This will greatly facilitate molecular breeding approaches for bioenergy crops.

EX2068.0008

Secondary Considerations: Industry Praise

Benjamino

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RESEARCH ARTICLE

Check for updates

Genome-Based Targeted Sequencing as a Reproducible Microbial Community Profiling Assay

© Jacquelyn Benjamino,* Benjamin Leopold

*The Jackson Laboratory for Genomic Medicine, Farmington, ME

ABSTRACT Current sequencing-based methods rely on marker gene (e.g., 16S rRNA) or metagenomic analysis. We present an approach based on a highly multiplexed oligonucleotide probe (microbial abundances from genome tagged forward, cost-effective microbiome profiling) 16S rRNA and mWGS strategies. The use of rigorous probe design criteria enabled robust To test the utility of the MA-GenTA assay, sequences representing bacteria present in the MA-GenTA data with mWGS data demonstrated 0.01% relative abundance and a similar number of sequences. Despite the incompleteness of the reference scaling (NMDS) clustering based on the 16S groups was consistent between MA-GenTA and mWGS. MA-GenTA represents a potentially useful method for profiling based on reference genomes.

IMPORTANCE New methods for profiling the approaches to understanding the composition of microbial communities. In this study, we combined bacterial genomic multiplexed single primer extension reaction communities, using stool from various mouse models. MA-GenTA, was benchmarked against genome sequencing methods and delivered high quality data. Since the probes were generated also able to provide functional pathway analysis of assayed samples. The method is more informative than metagenome shotgun sequencing.

KEYWORDS 16S, MA-GenTA, mWGS, microbial sequencing

The primary molecular methods for determining microbial community composition are based on marker gene sequencing or whole-genome sequencing. The 16S rRNA marker gene has been widely used for microbial community analysis across diverse ecosystems (1, 2). Using this method, taxonomic classification of the bacterial community can be obtained at modest cost and a resolution that ranges from subspecies to family level, depending on the 16S rRNA segment that is sequenced (4–6). Continued reduction in the cost of DNA sequencing has meant that mWGS approaches have become increasingly common due to the greater information on gene content, taxonomic resolution, and strain-level variation (7), despite higher cost and complexity of data analysis.

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species, strain, and even gene level. We adapted the Allegro Targeted Genotyping assay's single primer extension reaction that is widely used for genotyping (17, 18) and implemented it as a quantitative, straightforward, and cost-effective method for profiling mouse microbial communities based on the iMGMC hqMAGs.

EX2069.0002

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing **PATENT OWNER'S HEARING DEMONSTRATIVES** were served on January 16, 2026 and January 20, 2026 via email to Petitioner by serving the correspondence addresses of record as follows:

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