



ORAL ARGUMENT FOR IPR2025-00028 IPR2025-00029

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

Patent Owner's Hearing Demonstratives

February 11, 2026

The Petition Fails to Show Obviousness

▶ The Petition fails to show *prima facie* obviousness

- Shapero's array-based method is fundamentally different from NGS
- Delseny discourages modifying Shapero
- The cited art offers no motivation to combine

▶ Objective indicia reinforce the claims' non-obviousness

- The prior art discouraged and taught away from the claimed inventions
- The claimed inventions solved long-felt, unmet needs
- The industry praised Tecan's inventions
- QIAGEN copied Tecan's SPET
- QIAGEN praised Tecan's SPET
- QIAGEN published unexpected results



Shapero Published

Dec. 15, 2004

EX1006



Delseny Published

Aug. 3, 2010

EX1007

NGS Commercially Available

2007



Patent Priority Date

Jan. 26, 2012

EX1001



Shapero Authors Developed Affymetrix Hybridization Arrays

Shapero

MARA: a novel approach for high-density DNA oligonucleotide arrays

Michael H. Shapero*, Jane Zhang, Ann Loraine, Weiwei Liu, Xiaojun Di, Guoying Liu and Keith W. Jones

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Received October 8, 2004; Revised November 10, 2004; Accepted November 24, 2004

ABSTRACT

We have developed a locus-specific DNA target preparation method for highly multiplexed single nucleotide polymorphism (SNP) genotyping called MARA (Multiplexed Anchored Random Amplification). The approach uses a single primer per SNP in conjunction with restriction enzyme digested, adapter-ligated human genomic DNA. Each primer is composed of common sequence at the 5' end followed by locus-specific sequence at the 3' end. Following a primary reaction in which locus-specific products are generated, a secondary universal amplification is carried out using a generic primer pair corresponding to the oligonucleotide and genomic DNA adapter sequences. Allele discrimination is achieved by hybridization to high-density DNA oligonucleotide arrays. Initial multiplex reactions containing either 250 primers or 750 primers across nine DNA samples demonstrated an average sample call rate of ~95% for 250- and 750-plex MARA. We have also evaluated >1000- and 4000-primer plex MARA to genotype SNPs from human chromosome 21. We have identified a subset of SNPs corresponding to a primer conversion rate of ~75%, which show an average call rate over 95% and concordance >99% across seven DNA samples. Thus, MARA may potentially improve the throughput of SNP genotyping when coupled with allele discrimination on high-density arrays by allowing levels of multiplexing during target generation that far exceed the capacity of traditional multiplex PCR.

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MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays

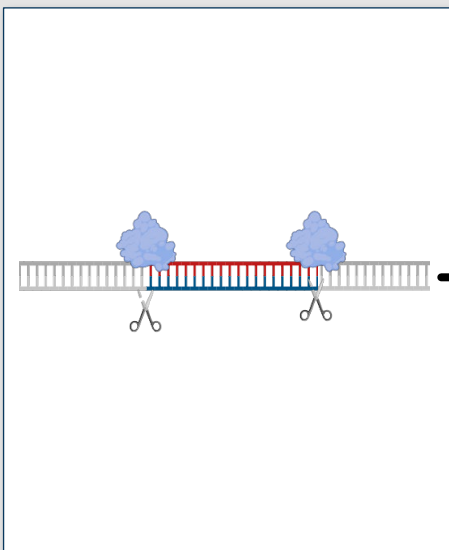
Michael H. Shapero*, Jane Zhang, Ann Loraine, Weiwei Liu, Xiaojun Di, Guoying Liu and Keith W. Jones

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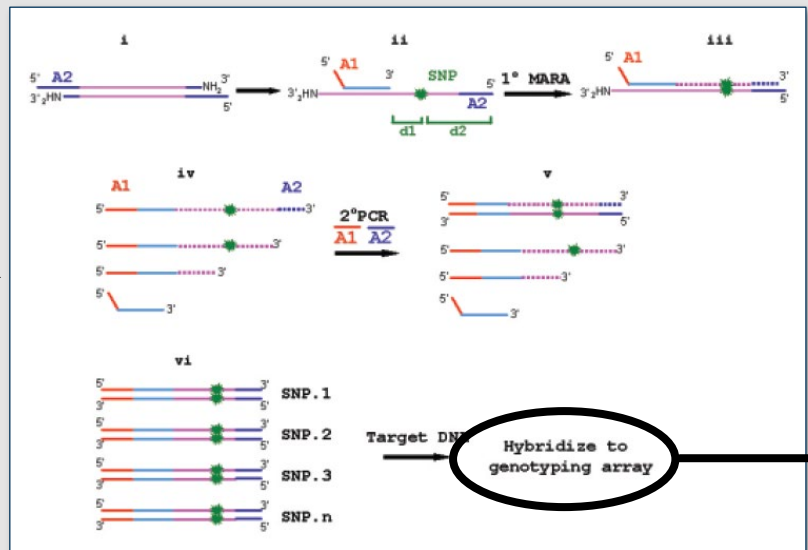
Received October 8, 2004; Revised November 10, 2004; Accepted November 24, 2004

EX1006.0001

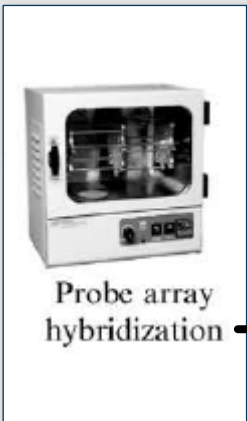
Shapero Described Optimized Method for Using Hybridization Arrays



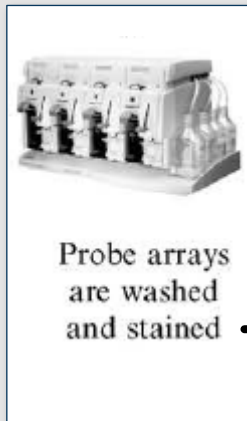
Site-Specific
Enzymatic
Digestion



MARA



Probe array
hybridization



Probe arrays
are washed
and stained

Table 1. 250- and 750-plex MARA

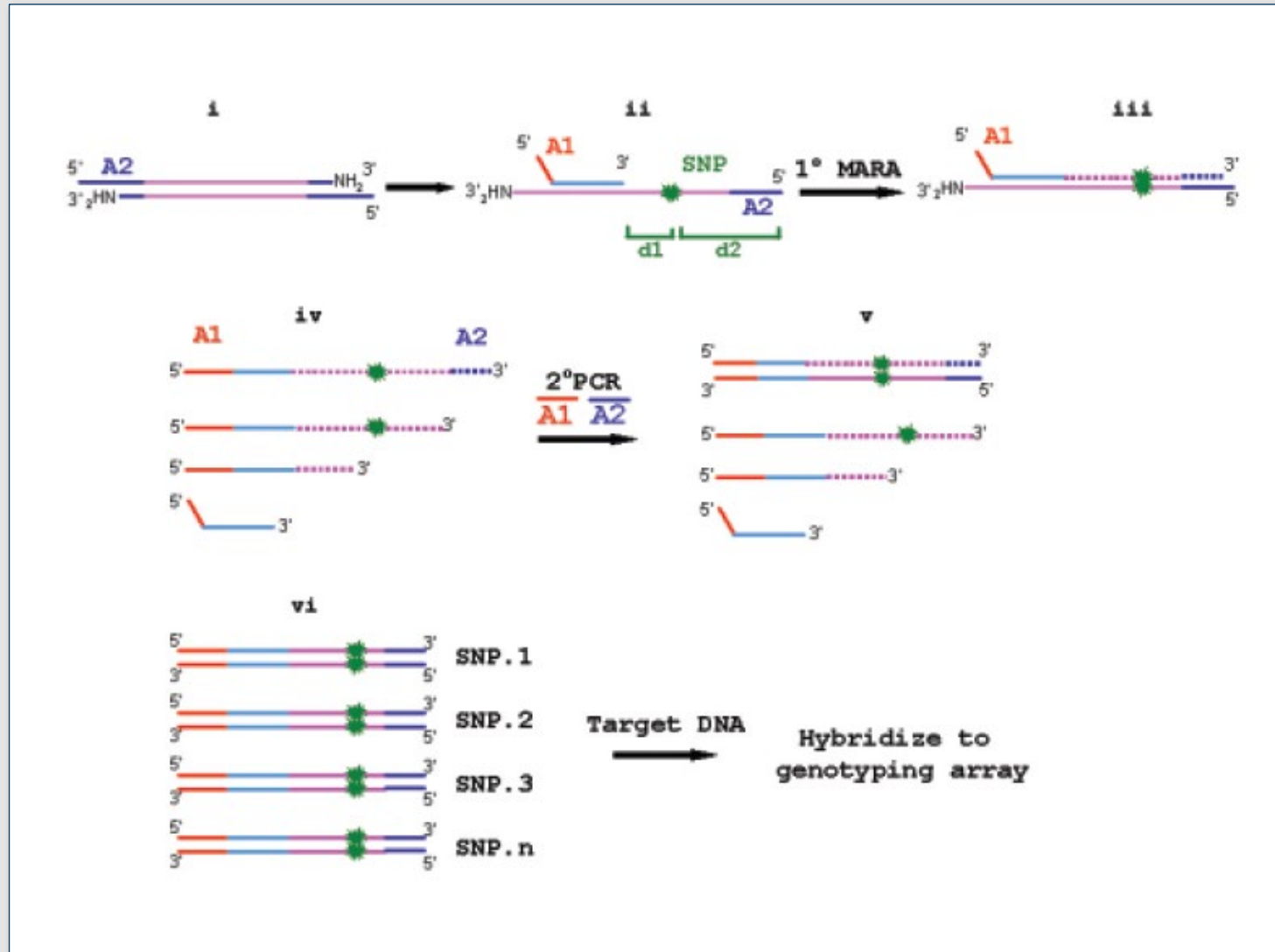
Sample	250-plex			750-plex		
	DS > 0.08 (%)	Call rate (%)	Concord (%)	DS > 0.08 (%)	Call rate (%)	Concord (%)
NA10848	100	97.5	96.7	99.4	94.8	97.2
NA12671	98.6	97.1	100	98.9	95.1	99.2
NA12672	99.6	97.1	97.8	99.6	95.0	98.2
NA04477	99.3	94.6	99.2	99.3	95.7	98.8
NA04479	98.9	94.6	97.7	98.5	95.7	98.4
NA05995	99.3	96.4	99.6	99.1	95.3	99.4
NA07057	100	97.1	98.5	99.3	95.9	98.4
NA10830	98.9	94.6	99.2	99.1	96.1	99.4
NA10831	100	97.1	98.5	98.3	95.0	98.8
Average	99.4	96.2	98.6	99.0	95.4	98.7

DS of a probe pair is the ratio of (PM - MM)/(PM + MM), where PM is the intensity of the perfect match cell and MM is the intensity of the mismatch cell. The DS threshold of 0.08 is higher than the threshold value of 0.03 used in WGA as a detection filter to identify weak hybridization signals (31). "Concord" refers to the concordancy of MARA genotype calls with WGA genotype calls.

Array Data Output
and Analysis

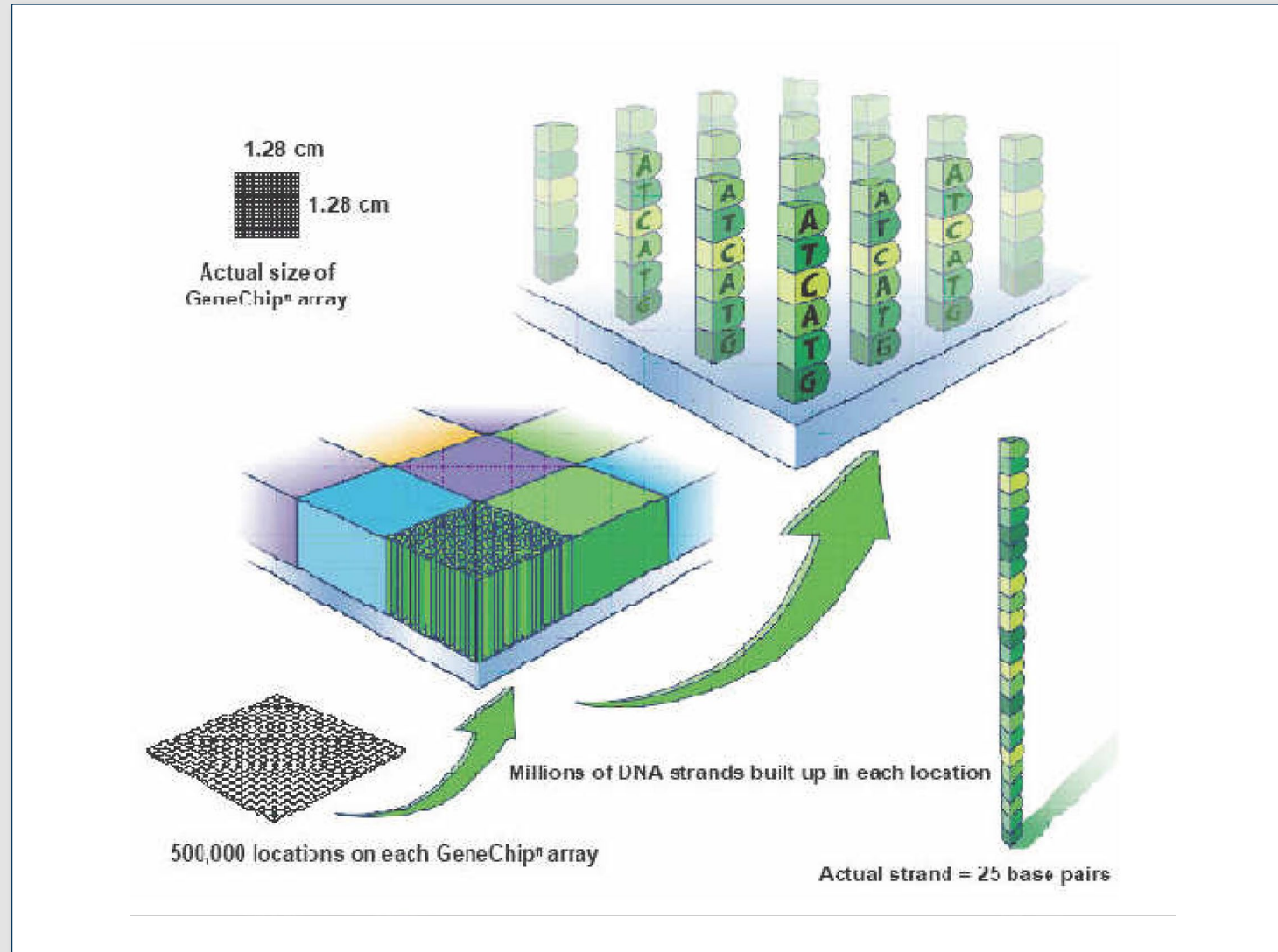
EX1006.0002-04

Shapero's MARA Library Preparation Method



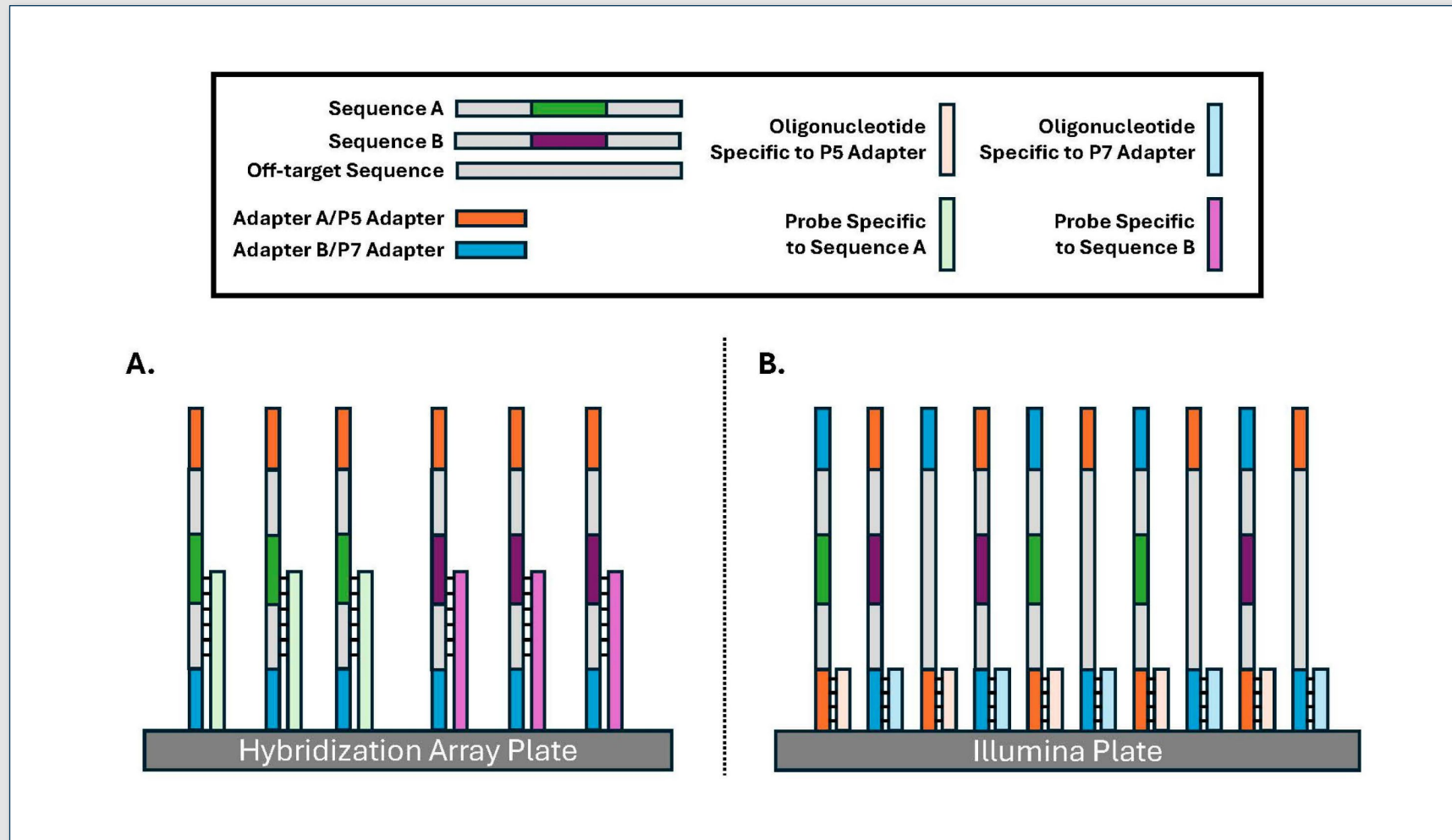
EX1006.0004

Shapero Used Affymetrix GeneChip® Hybridization Arrays



EX2003.0001

Hybridization Arrays Are Target-Specific; NGS Is Not



EX2107.¶112

A POSA Would Have Understood the Importance of Specificity



**Carlos Bustamante,
Ph.D.**

285. The methods claimed in the '012 and '108 patents disclose the use of a single target-specific primer to generate oligonucleotide extension products containing a sequence of interest and the amplification of the oligonucleotide extension products to generate enriched sequences of interest that can be sequenced through NGS. In my opinion, as of 2012, a POSA would have been aware of the nearly two decades worth of literature that demonstrated concerns with amplification, such as target specificity and off-target binding. *See supra* ¶¶ 57-62. Additionally, a POSA would have been aware from literature concerning the development and improvement of NGS library preparation methods that the need for accurate target specificity was paramount. *See supra* ¶¶ 87-104.

EX2107.¶285

Shapero Relies on the Combination of MARA With the Hybridization Arrays

MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays

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Long before the completion of the human genome sequence, it was clear that sites of genetic variation could be used as markers to identify disease segregating patterns among families (1). This approach led to the identification of a number of genes involved in rare, monogenic disorders. With the complete sequence now available (2,3), attention has turned to the challenge of identifying common disease genes. Although

multiple sources of genetic variation occur among individuals, single nucleotide polymorphisms (SNPs) are emerging as the marker of choice for genetic association studies due to their abundance, stability and relative ease of scoring. Additionally, the ongoing international effort to build a haplotype map will identify a standard set of common-allele SNPs that are expected to provide the framework for new genome-wide studies designed to identify the underlying genetic basis of complex diseases, pathogen susceptibility and differential drug responses (4). Owing to these wide-ranging applications, there is still a need for the development of robust, flexible, cost-effective technology platforms for scoring SNP genotypes in large numbers of samples.

There are a multitude of genotyping methodologies for SNPs that have been described over the years (5–7). These approaches can vary in terms of how alleles are discriminated, e.g. the use of nucleic acid hybridization with short oligonucleotide probes alone or in combination with enzymes such as DNA polymerases or DNA ligases. Additionally, these approaches can vary in the nature of both the assay formats and labeling and detection strategies. However, many currently used approaches share a common feature in that there is a requirement for an amplification of the region harboring the polymorphism prior to actual genotyping. PCR significantly increases the copy number of the sequence to be genotyped, thereby effectively reducing the complexity of the target sequences and improving the ability to differentiate correct and incorrect genotype calls. Traditionally, standard multiplex PCR is difficult to design and enable at levels beyond several dozen loci per reaction (8). Even with careful optimization of primer regions (9) and efforts to minimize non-specific primer dimers (10), multiplex PCR is not a routine laboratory technique. A number of alternative approaches to standard multiplex PCR have been described over the years. These include the use of universal primer sequences added to the ends of locus-specific sequences (11), the use of PCR suppressors in which one target-specific primer is used in conjunction with a means common to all targets present on all DNA fragments (12,13) and the use of acrylamide spheres with covalently attached primers for solid-phase PCR amplification (14). While these methods do improve multiplexing levels in comparison with standard approaches, none of the described methods has demonstrated reactions >100-plex and thus are still of moderate utility for a high-throughput

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templates due to product length differences. Importantly, MARA does not require absolute specificity of primer annealing and elongation since target recognition and allele discrimination is achieved through hybridization to high-density arrays. Following the secondary amplification, the preparation

EX1006.0003-04

In conclusion, multiplex levels for SNP genotyping that are higher than typically achieved through standard multiplex PCR have been demonstrated using MARA in conjunction with allele-specific hybridization on high-density DNA arrays.

EX1006.0008

Petitioner Focuses Solely on MARA, Divorced From Arrays

MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density arrays

Michael H. Shapiro
Guoying Liu and

Affymetrix, Inc., Gen

Received October 8, 2004

ABSTRACT

We have developed a novel method for preparing nucleic acid arrays for high-density SNP genotyping. The approach uses a combination of adapter-ligated beads and high-density arrays. Following a primer extension, the products are hybridized to the arrays. The method is carried out in a single reaction. The approach is applicable to a wide range of samples and has been used to genotype over 1000 SNPs in a single reaction. The approach is applicable to a wide range of samples and has been used to genotype over 1000 SNPs in a single reaction. The approach is applicable to a wide range of samples and has been used to genotype over 1000 SNPs in a single reaction.

INTRODUCTION

Long before the completion of the Human Genome Project, it was clear that the identification of disease-associated genes was a major challenge. The identification of disease-associated genes was a major challenge. The identification of disease-associated genes was a major challenge.

DNA samples. These results suggest that MARA may serve as a target preparation method for highly multiplexed locus-specific SNP genotyping that far exceeds the capacity of standard traditional multiplex PCR. When coupled with a method for allelic discrimination such as high-density array hybridization, this method provides access to large numbers of locus-specific SNPs in a single reaction and thus may facilitate the use of SNPs in genome-wide association studies.

**PETITIONER
CITES**

Paper 22, 5-6

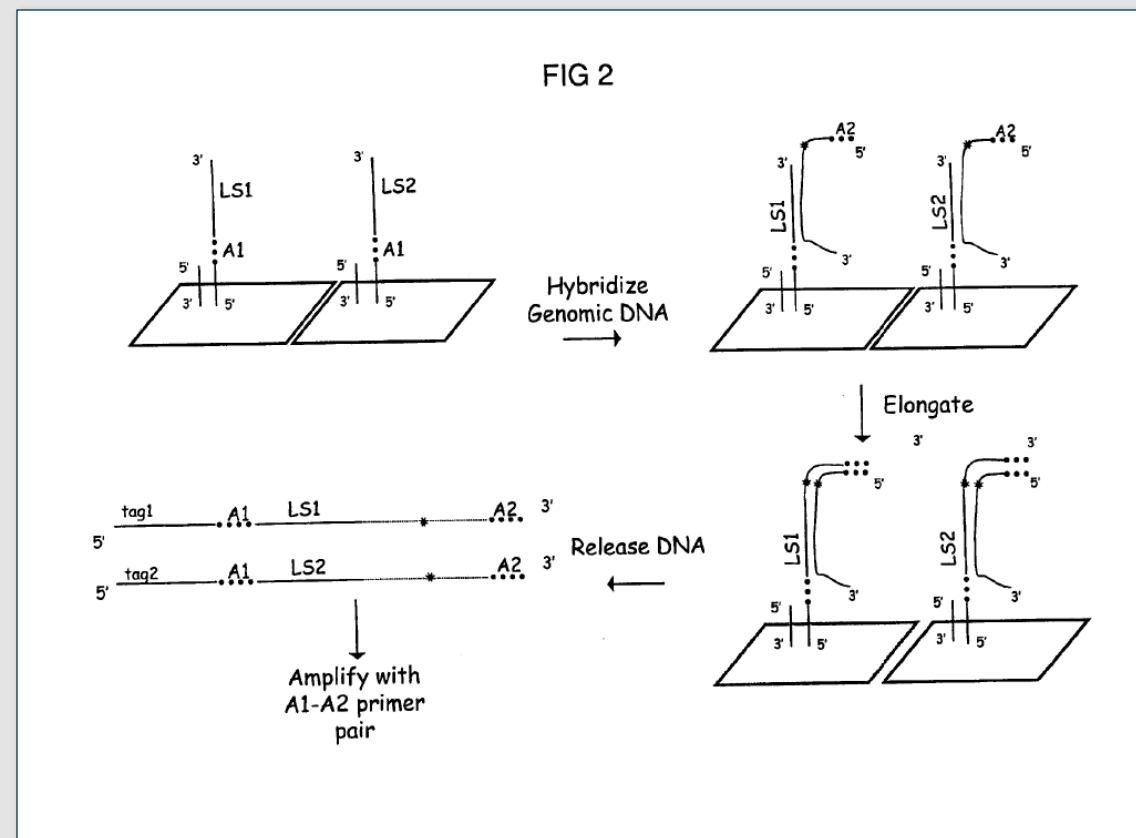
EX1006.0002

IPR2025-00028: Paper 27, 5-6; IPR2025-00029: Paper 27, 5-6

Shapero and Jones Never Separate MARA From Arrays

[0080] A second embodiment of the methods is illustrated in FIG. 2. Capture probes are designed with a locus specific region (LS1 or LS2) and a common sequence (A1) as in FIG. 1. In this embodiment the capture probes further comprise a tag sequence that is unique for each species of capture probe designed. (For a description of tags and tag probes, see, U.S. Ser. No. 08/626,285.) The capture probes are attached to the array through hybridization of the tag sequence to a substantially complementary tag probe sequence that is attached to the array. The tag probes may be attached to the array in discrete locations. Different species of tag probes are present at different discrete, spatially addressable locations. Adaptor-ligated genomic DNA is hybridized to the array so that the capture probes hybridize to target sequences in the sample. The capture probes are extended as in FIG. 1 to incorporate the target sequence and common sequence A2. The extended capture probes are released and amplified using primers A1 and A2. The amplified product may then be analyzed by, for example, hybridization to an array. Information about the region of interest can be determined by analysis of the hybridization pattern. The amplified sample may be analyzed by any method known in the art, for example, MALDI-TOF mass spec, capillary electrophoresis, OLA, dynamic allele specific hybridization (DASH) or TaqMan® (Applied Biosystems, Foster City, Calif.). For other methods of genotyping analyses see Syvanen, *Nature Rev. Gen.* 2:930-942 (2001) which is herein incorporated by reference in its entirety.

EX1011.¶80



EX1011

PETITIONER CITES

Paper 22, 16

IPR2025-00028: Paper 17, 35-36; Paper 27, 6; IPR2025-00029: Paper 17, 35-36; Paper 27, 6; EX2107.¶¶170-173, 234-236

Shapero's Data Is Based on Use of Array Technology

Table 1. 250- and 750-plex MARA

Sample	250-plex			750-plex		
	DS > 0.08 (%)	Call rate (%)	Concord (%)	DS > 0.08 (%)	Call rate (%)	Concord (%)
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the primers were not. As explained above, Shapero relies on the call rates coupled with discrimination scores ("DS") to evaluate the percentage of SNPs that were detected by the hybridization arrays. *See supra*, ¶¶ 162-64. The data is wholly obtained through the use of hybridization arrays and not NGS platforms. *See supra*, ¶¶ 150, 162-64. Given the fact that target specificity was driven by the hybridization arrays, a POSA could not infer from the data what degree of target specificity was driven by the MARA method, let alone how that method might translate to use with NGS where there are not safeguards to ensure target specificity.

EX2107.¶220

EX1006.0005

Shapero's Gel Reads Do Not Demonstrate Specificity

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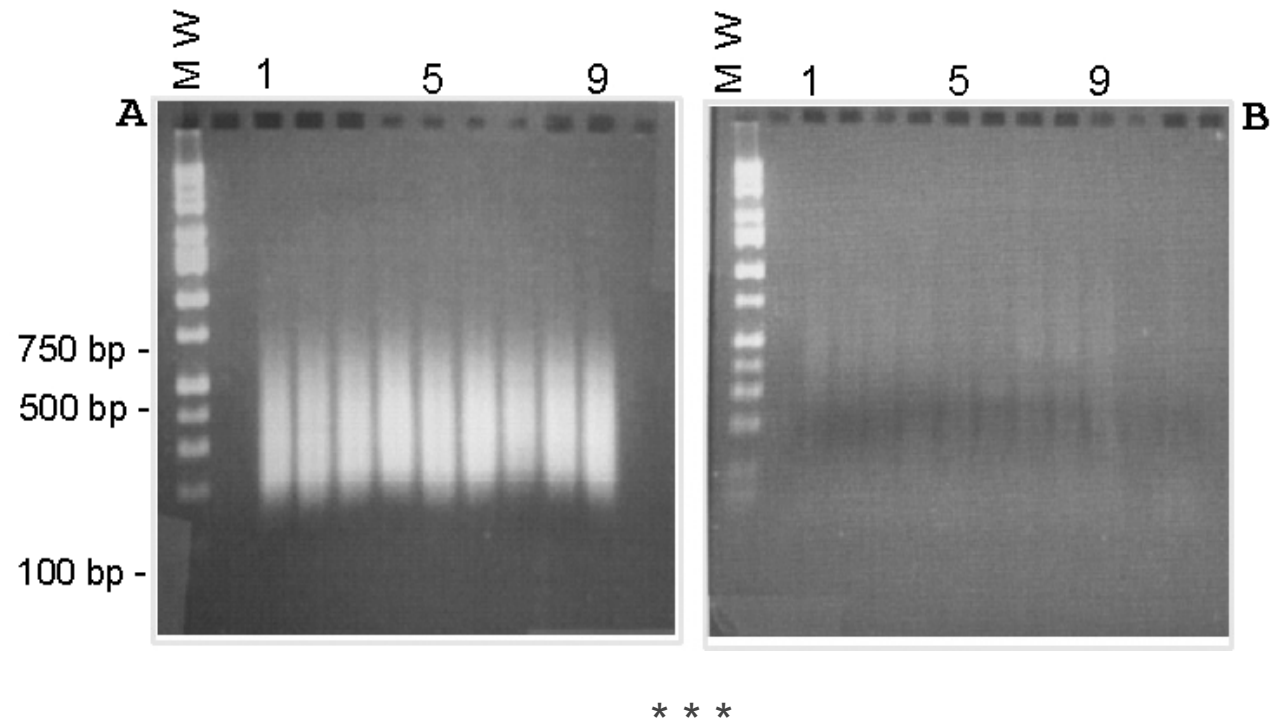


Figure 2. (A) 250-plex 2° PCR products separated on a 2%, 1× TBE agarose gel. (B) Negative control (no locus-specific primers) 2° PCR products separated on a 2%, 1× TBE agarose gel. (C) p502 chip image using 250 primer-plex 2° PCR products as target derived from input genomic DNA NA10830 (D) p502 chip image using negative control 2° PCR products as target derived from input genomic DNA NA10830.

EX1006.0004

IPR2025-00028: Paper 27, 11-13; IPR2025-00029: Paper 27, 11-13; see also EX1120.0213:10-216:2, 348:12-350:9

Shapero's Negative Controls Assess Amplification, Not Specificity

MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays

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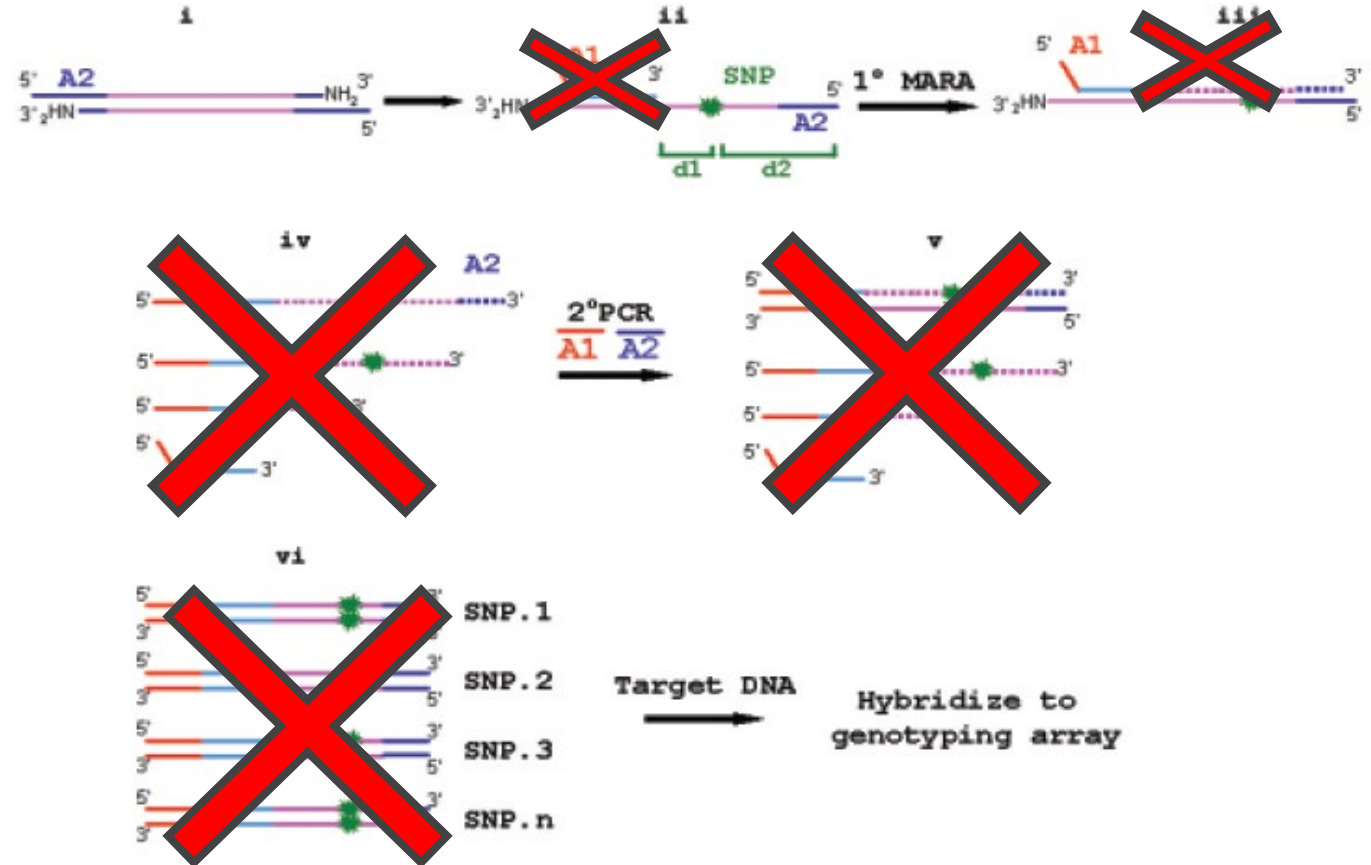
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There are a multitude of genotyping methodologies for SNPs that have been described over the years (5-7). These approaches can vary in terms of how alleles are discriminated, e.g. the use of nucleic acid hybridization with short oligonucleotide probes alone or in combination with enzymes such as DNA polymerases or DNA ligases. Additionally, these approaches can vary in the nature of both the assay formats and labeling and detection strategies. However, many currently used approaches share a common feature in that there is a requirement for an amplification of the region harboring the polymorphism prior to actual genotyping. PCR significantly increases the copy number of the sequence to be genotyped, thereby effectively reducing the complexity of the target sequences and improving the ability to discriminate correct and incorrect genotype calls. Traditionally, standard multiplex PCR is difficult to design and enable at levels beyond several dozen loci per reaction (8). Even with careful optimization of repeat regions (9) and efforts to minimize non-specific primer dimers (10), multiplex PCR is not a routine laboratory technique. A number of alternative approaches to standard multiplex PCR have been described over the years. These include the use of universal primer sequences added to the ends of locus-specific sequences (11), the use of PCR suppressors in which one target-specific primer is used in conjunction with a means common to all targets present on all DNA fragments (12,13) and the use of acrylamide spheres with covalently attached primers for solid-phase PCR amplification (14). While these methods do improve multiplexing levels as compared with standard approaches, none of the described methods has demonstrated reactions >100-plex and thus are still of moderate utility for a high-throughput

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ized in Table 1. A total of 99.4% of the SNPs targeted by locus-specific primers across nine individuals had a mean DS > 0.08. The targeted SNPs had a mean call rate of 96.2%, and mean concordance of 98.6% to WGA genotype calls. In contrast, when locus-specific primers were omitted in the primary reaction, 6.6% (of the 258 SNPs) had a mean DS > 0.08. There was a mean call rate of 6.3%; however, only five of these SNPs were called across at least five of the nine DNA samples. These results show that the presence of locus-specific primers allows specific target sequences to be amplified, and in the absence of primers, only small quantities of non-specific products are generated.

EX1006.0005

Dr. Metzker Concedes That Amplification Does Not Occur Without the Locus-Specific Primers



**Michael Metzker,
Ph.D.**
Petitioner's Expert

amino block helps to prevent background genomic amplification.”⁵⁵ Shapero
(2004)’s common primers will not produce amplification products on their own
independent of the target specific primer extension through the A2 adapter.⁵⁶ Siebert

EX1121.¶23

would be off-target events. Because those common primers did not produce
quantities sufficient for the chip to detect, this demonstrates to one of ordinary skill

EX1121.¶19

Dr. Bustamante Confirmed the Data Does Not Show Specificity



**Carlos Bustamante,
Ph.D.**

There is no actual evidence presented in the paper that would show a POSA that the primers that are used in the 250 plex reaction, as reported in QIAGEN 1006, the Shapero reference, are binding at a relatively high off-target rate, correct?

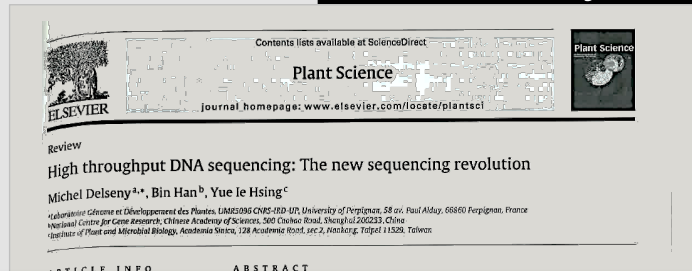
Q. Yes or no?

A. There is no evidence either way.

EX1120.0134:11-22

Delseny Reviews NGS Technologies

Delseny



High throughput DNA sequencing: The new sequencing revolution

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QIAGEN Exhibit 1007

EX1007.0407

IPR2025-00028: Paper 17, 1-2; Paper 27, 6; IPR2025-00029: Paper 17, 1-2; Paper 27, 6; EX2107.¶¶148-149

Delseny Provides No Motivation to Arrive at Challenged Claims

Delseny

So far, genotyping has involved mostly microsatellites, a technology with limited resolution and requiring much time and human resources. The ultimate resolution is at the base-pair level and NGSTs now open the possibility to genotype a line by sequencing it, provided a reference genome is available. This strategy has recently been illustrated in rice [125] in total 150 RILs derived from a cross between 93-11 × Nipponbare, (the two indica and japonica rice reference genomes) were re-sequenced with use of the Illumina machine and the barcode technology. With this multiplexing, the sequencing of the 150 lines all, 1,500,000 SNPs were detected, recombination points (33.3 ~35× higher than that average time of acquisition of data is 20× faster than with previous methods. Such an approach can substitute for the genotyping microarray strategy because of its higher resolution.

[125] X. Huang, Q. Feng, Q. Qian, et al., High-throughput genotyping by whole genome sequencing, *Genome Res.* 19 (2009) 1068–1076.

EX2117 (Han)

EX1007.0417, 421

Han Criticizes Methods Like MARA

Han

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Methods

High-throughput genotyping by whole-genome resequencing

Xuehui Huang,^{1,6} Qi Feng,^{1,2,6} Qian Qian,^{3,6} Qiang Zhao,^{1,2,6} Lu Wang,^{1,6} Ahong Wang,^{1,6} Jianping Guan,¹ Danlin Fan,¹ Qijun Weng,¹ Tao Huang,¹ Guojun Dong,³ Tao Sang,^{1,4} and Bin Han^{1,5,7}

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The next-generation sequencing technology coupled with the growing number of genome sequences opens the opportunity to redesign genotyping strategies for more effective genetic mapping and genome analysis. We have developed a high-throughput method for genotyping recombinant populations utilizing whole-genome resequencing data generated by the Illumina Genome Analyzer. A sliding window approach is designed to collectively examine genome-wide single nucleotide polymorphisms for genotype calling and recombination breakpoint determination. Using this method, we constructed a genetic map for 150 rice recombinant inbred lines with an expected genotype calling accuracy of 99.94% and a resolution of recombination breakpoints within an average of 40 kb. In comparison to the genetic map constructed with 287 PCR-based markers for the rice population, the sequencing-based method was ~20× faster in data collection and 35× more precise in recombination breakpoint determination. Using the sequencing-based genetic map, we located a quantitative trait locus of large effect on plant height in a 100-kb region containing the rice "green revolution" gene. Through computer simulation, we demonstrate that the method is robust for different types of mapping populations derived from organisms with variable quality of genome sequence and is feasible for organisms with large genome sizes and low polymorphisms. With continuous advances in sequencing technologies, this genome-based method may replace the conventional marker-based genotyping approach to provide a powerful tool for large-scale gene discovery and for addressing a wide range of biological questions.

Supplemental material is available online at www.genome.org. Pseudomolecules harboring 1,226,791 SNPs identified between *Oryza sativa* ssp. *indica* cv. 9311 and ssp. *javanica* cv. Nipponbare are available at <http://www.ncgr.ac.cn/english/edatabase.htm>. The raw Illumina sequencing data are available in the EBI European Nucleotide Archive (<http://ftp.ebi.ac.uk/>) with accession number ERA000078.

The first use of DNA-based markers decades ago laid the groundwork for gene discovery through forward and reverse genetics. The types of markers and methods for constructing genetic maps have evolved rapidly with advances in molecular biology techniques. The development of PCR triggered the burst of a generation of markers that considerably simplified experimental procedures for marker designing and scoring. However, these markers, although still widely used, have shown growing limitations in chromosomal coverage, time, and cost effectiveness. The development of genomic sciences and tools has set the stage for replacing the marker-based mapping approach with genome-based high-throughput strategies.

The availability of genome sequences opened the door to high-throughput genotyping. This was initially accomplished by adopting microarray technology, which detects single nucleotide polymorphisms (SNPs) through hybridizing genomic DNA to oligonucleotides spotted on gene chips. This genotyping method

substantially improved the efficiency of marker collection by allowing the detection of hundreds to thousands of markers in a single hybridization (Winkler et al. 1998). It has been applied to model systems such as human, *Arabidopsis*, and rice (Medzian et al. 2006; Stiger et al. 2006; Jeremy et al. 2009). Although the goal of high-throughput was achieved, serious limitations remain for the array-based method. It is laborious, time-consuming, and expensive to design, produce, and process microarrays suited for specific mapping populations.

The advent of the next-generation sequencing technology holds the promise for a methodological leap forward in genotyping and genetic mapping. The new sequencing techniques not only increase sequencing throughput by several orders of magnitude but also allow simultaneously sequencing a large number of samples using a multiplexed sequencing strategy (Jiang et al. 2008; Crum et al. 2009). These nascent technical advances have paved the way for the development of a sequencing-based high-throughput genotyping method that combines advantages of time and cost effectiveness, dense marker coverage, high mapping accuracy and resolution, and more compatible genome and genetic maps among mapping populations and organisms.

Here we describe the first high-throughput genotyping method that uses SNPs detected by whole-genome resequencing.

Here we describe the first high-throughput genotyping method that uses SNPs detected by whole-genome resequencing.

SNP site. Second, an individual SNP site is no longer a reliable marker or locus for genotyping due to several potential sources of sequence errors. To deal with these unique features of the SNP data

Genotyping with various types of molecular genetic markers has long been a laborious and time-consuming step that limited the power and efficiency of genetic analyses and gene discovery. Here we show

1068 Genome Research

19-1058-1076 © 2009 by Cold Spring Harbor Laboratory Press; ISSN 1068-7021/09; www.genome.org

Tecon Ex. 2117, Page 1 of 10

QIAGEN Sciences, LLC v. Tecon Genomics - IPR2025-00028

EX2117.1068-69, 73

IPR2025-00028: Paper 17, 32-33; Paper 27, 15-16; IPR2025-00029: Paper 17, 32-33; Paper 27, 15-16; EX2107.¶¶191-196, 238-243

Petitioner Fails to Show a Motivation to Combine



KSR did not do away with the requirement that there must exist a motivation to combine various prior art references in order for a skilled artisan to make the claimed invention.

Virtek Vision Int'l ULC v. Assembly Guidance Sys., Inc., 97 F.4th 882, 887 (Fed. Cir. 2024)



Obviousness concerns whether a skilled artisan not only *could have made* but *would have been motivated to make* the combinations or modifications of prior art to arrive at the claimed invention.

Virtek Vision Int'l ULC, 97 F.4th at 886-87 (emphases original) (cleaned up)

Objective Indicia Reinforce Non-Obviousness

- ▶ Prior Art Discouraged and Taught Away from Claimed Inventions
- ▶ The Claimed Inventions Solved Long-Felt, Unmet Needs
- ▶ The Industry Praised Tecan's Inventions
- ▶ QIAGEN Copied Tecan's SPET
- ▶ QIAGEN Praised Tecan's SPET
- ▶ QIAGEN Published Unexpected Results

The Prior Art Discouraged the Claimed Methods

- | | | |
|---------------------------------|------------------------------|---------------------------------|
| 1. Syvänen (EX1012) | 10. Kozarewa (EX2054) | 19. Myllykangas (EX2052) |
| 2. Delseny (EX1007) | 11. Aird (EX2042) | 20. Grunenwald (EX2056) |
| 3. Han (EX2117) | 12. Metzker (EX1025) | 21. Palka-Santini (EX2139) |
| 4. Siebert (EX1101) | 13. Devon (EX2044) | 22. Bustin (EX2018) |
| 5. Miura (EX2047) | 14. Padegima (EX2045) | 23. Bui (EX2138) |
| 6. Tsoktouridis (EX2048) | 15. Arnold (EX2046) | 24. Howell (EX2130) |
| 7. Sohn (EX2050) | 16. Bower (EX2049) | 25. Moorthie (EX2169) |
| 8. Quail 2008 (EX2053) | 17. Bronner (EX2155) | 26. Ronaghi (EX2129) |
| 9. Quail 2011 (EX2071) | 18. Almomani (EX2051) | |

The Prior Art Discouraged Use of Key Aspects of the Claimed Inventions

Single Target-Specific Primer

- | | |
|-----------------------------|-------------------------|
| 1. Delseny
(EX1007) | 6. Sohn
(EX2050) |
| 2. Han
(EX2117) | 7. Devon
(EX2044) |
| 3. Siebert
(EX1101) | 8. Pdegimas
(EX2045) |
| 4. Miura
(EX2047) | 9. Arnold
(EX2046) |
| 5. Tsoktouridis
(EX2048) | 10. Bower
(EX2049) |

PCR Amplification

- | | |
|-----------------------------|-----------------------------|
| 1. Syvänen
(EX1012) | 7. Metzker
(EX1025) |
| 2. Tsoktouridis
(EX2048) | 8. Bronner
(EX2155) |
| 3. Quail 2008
(EX2053) | 9. Almomani
(EX2051) |
| 4. Quail 2011
(EX2071) | 10. Myllykangas
(EX2052) |
| 5. Kozarewa
(EX2054) | 11. Grunenwald
(EX2056) |
| 6. Aird (EX2042) | |

Siebert Discouraged Claimed Use of a Single Primer

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, KpnI, DpnI, PstI and SmaI 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 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



Figure 1. (A) Sequences of the walking adaptor and adaptor primers and gene-specific primers. (B) Illustration of the suppression PCR effect. Structures 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

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.

EX1101.1087-88

Siebert Discouraged Claimed Amplification

An improved PCR method for walking in uncloned genomic DNA

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

Clontech,
Ovchinnik
Moscow
Received 31

Several PCR known re genomic D randomly However, walking is complicate methods b however, We have PCR (9,1) examined method b (5), which attempted plasmidog character. In our h PCR gene performed were found multiple P without Se We have combining termed 'su ends of h genomic D Following as a templ primers. The seq in Figure to both ex enzymes of restriction (blunt end pBluescript The 've amine gro

*To whom

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

A
Adaptor 5'-CTA ATA CGA CTC ACT ATA GGG CTC GAG CCG CCG CCC GGG CAG GT-3'
AP1 5'-GGATC CTA ATA CGA CTC ACT ATA GGG C-3' 3'-H2N-CCC GTC CA-P-5'
AP2 5'-A ATA GGG CTC GAG CCG C-3'

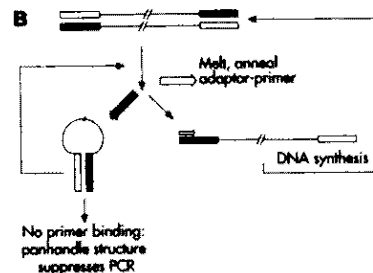


Figure 1. (A) Sequences of the walking adaptor and adaptor primers and gene-specific primers. **(B)** Illustration of the suppression PCR effect. Structure of PCR products formed by non-specific amplification. The sequences on the ends of the products contain inverted terminal repeats and will form 'panhandle'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

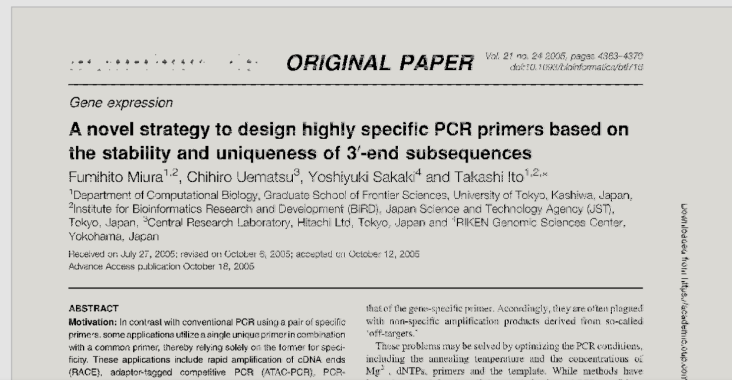
Primary PCR reactions were conducted in 50 μ l volumes containing 1 μ l of ligated and diluted DNA, 40 mM Tris-HCl, pH 9.3, 85 mM KOAc, 1.1 mM MgOAc, 0.4 μ M adaptor primer AP1 and gene-specific primer, and 0.9 μ l of Tth/Vent polymerase/antibody mixture. The cycle parameters were as follows: denaturation at 94°C for 30 s and annealing/extension at 68°C for 6 min, except for an initial denaturation step of 1 min and a final annealing/extension time of 15 min, 35 cycles of PCR were used. A secondary PCR reaction was conducted with 1 μ l of a 100-fold dilution of the primary PCR using adaptor primer AP2 and the nested gene-specific primer. The same reaction composition and cycle parameters, except that 0.6 μ l of the Tth/Vent polymerase/antibody mixture was used and 20 thermocycles performed. PCR products were examined on 1% agarose/EtBr gels. For restriction enzyme digestion of PCR products, 10 μ l of PCR products were mixed with 1.2 μ l of 10 $\times$ enzyme buffer and 10 U restriction enzyme (Life Technologies) and incubated for 4 h at 37°C.

EX1101.1087

EX1101.1088

28

Miura Confirmed Problems With Single Primer Methods



Miura

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.

The results of the PCR are shown in Figure 2B. Although all the primers allowed us to obtain PCR products from the adaptor-tagged genomic DNA template, the yield and purity of the bona fide target products are substantially different between the primers. Some PCR products were obtained in high yield and purity, but others were obtained in low yield and were plagued with many off-target products. Notably, all the primers shared a comparable efficiency and specificity in amplification when used in combination with a reverse primer specific to *PDR3* (Pdr3-r) (Fig. 2C). It thus appears that the difference in the specificity of each primer led to variable product yields and purities.

* * *

mer performance tended to be. Although the correlation was not complete, we can roughly predict the performance of the primer based on the uniqueness of the SDSS and can, at least, eliminate apparently poor primers.

EX2047.4366;
see also EX2047.4369

EX2047.4363

IPR2025-00028: Paper 17, 41-44; Paper 27, 9-10, 21-22; IPR2025-00029: Paper 17, 41-44; Paper 27, 9-10, 21-22; EX2107.¶¶¶61-62, 248-254, 285-287

The Prior Art Discouraged Claimed Use of a Single Primer

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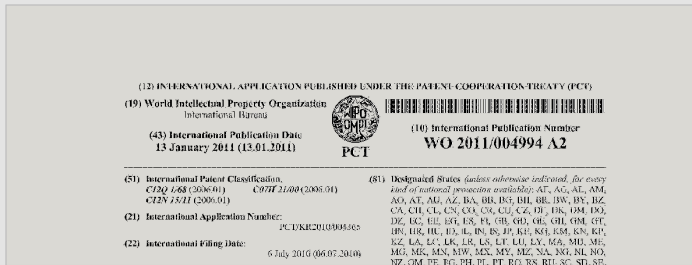
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(54) Title: A NOVEL GENOME WALKING METHOD FOR CLONING OF UNKNOWN DNA SEQUENCES ADJACENT TO A KNOWN SEQUENCE

(57) Abstract: The present invention relates to a novel genome walking method for cloning unknown sequences adjacent to a known sequence 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) cloning a genomic DNA containing a known nucleotide sequence adjacent to an unknown nucleotide sequence using a restriction enzyme; 2) cloning the genomic DNA with a specific primer; 3) finding the terminal sequence of the cloned DNA; 4) 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.

Tecan Ex. 2050, Page 1 of 19
IDT, Inc. v. Tecan Genomics - IPR2025-00016

Sohn Is Strong Evidence of Non-Obviousness



Sohn

(57) Abstract: The present invention relates to a novel genome walking method for cloning unknown genomic regions adjacent to known sequences by PCR. More particularly, the present invention relates to a method for cloning an unknown nucleotide sequence adjacent to a known nucleotide sequence, comprising the steps of 1) cleaving a genomic DNA that contains a known nucleotide sequence and an unknown nucleotide sequence adjacent thereto using a restriction enzyme; 2) modifying the 3'-OH end of the cleaved DNA with a nucleotide analogue; 3) linking the terminal sequence of the cleaved DNA fragment with a linker, adapter or cassette having a terminal sequence complementary thereto; and 4) 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.

IDT, Inc. v. Tecan Genomics - IPR2025-00016

EX2050.0001

IPR2025-00028: Paper 17, 41-44; Paper 27, 21-22; IPR2025-00029: Paper 17, 41-44; Paper 27, 21-22; EX2107.¶¶61-62, 248-254, 285-287

Syvänen Teaches Away From Claimed Amplification

Future trends

In practice, the requirement of a PCR amplification step to achieve sensitive and specific SNP genotyping is the principal factor that limits the throughput of assays today. Multiplex PCR amplification of more than ten DNA fragments is difficult to carry out reproducibly owing to the generation of spurious amplification products^{39,41,42,80}.

EX1012.0939

ACCESSING GENETIC VARIATION: GENOTYPING SINGLE NUCLEOTIDE POLYMORPHISMS

Ann-Christine Syvänen

Understanding the relationship between genetic variation and biological function on a genomic scale is expected to provide fundamental new insights into the biology, evolution and pathophysiology of humans and other species. The hope that single nucleotide polymorphisms (SNPs) will allow genes that underlie complex disease to be identified, together with progress in identifying large sets of SNPs, are the driving forces behind intense efforts to establish the technology for large scale analysis of SNPs. New genotyping methods that are high throughput, accurate and cheap are urgently needed for gaining full access to the abundant genetic variation of organisms.

LINEARLY POLYMERIZING
AMPLIFYING
Amplification of a single
nucleotide polymorphism
(SNP) by PCR. The
amplification product is
then sequenced to
determine the SNP
genotype.

Comparison of genomic DNA sequences in different individuals reveals some positions at which two, or in some cases more than two, bases can occur. These single nucleotide polymorphisms (SNPs) are highly abundant, and are estimated to occur at 1 out of every 1,000 bases in the human genome¹⁴. Depending on where a SNP occurs, it might have different consequences at the phenotypic level. SNPs in the coding regions of genes that alter the function or structure of the encoded proteins are a necessary and sufficient cause of most of the known recessively or dominantly inherited monogenic disorders. These SNPs are routinely analysed for diagnostic purposes. Another important group of SNPs are those that alter the primary structure of a protein involved in drug metabolism. These SNPs are targets for pharmacogenetic analysis¹⁵. Missense SNPs in the coding regions of genes, such as the two SNPs in the *cytochrome p450 2D6* gene and the *factor V Leiden* mutation¹⁶, can also contribute to common disease. This type of SNP can be analysed to assess the risk of an individual for a particular disease. In addition, it is likely that SNPs in the regulatory regions of genes might influence the risk of common disease. However, most SNPs are located in non-coding regions of the genome, and have no direct known impact on the phenotype of an individual.

These SNPs are useful as markers in population genetics and evolutionary studies¹⁷.

The reason for the current enormous interest in SNPs is the hope that they could be used as markers to identify genes that predispose individuals to common, multifactorial disorders by using microarray technology¹⁸. The rationale would be to genotype a collection of SNPs that occur at regular intervals and cover the whole genome to detect genomic regions in which the frequencies of the SNP alleles differ between patients and controls. It is assumed that the SNPs alleles are inherited together with the disease-predisposing alleles through the generations because they are physically close to each other. The disease-predisposing genes could then be localized and isolated, and protein-coding byproducts would be valuable targets for developing new therapeutic drugs. As a result of the efforts of the SNP Consortium—a collaboration of 14 major pharmaceutical companies and the Wellcome Trust, as well as members of the Human Genome Project¹⁹—there are almost 2 million SNPs in public databases, and perhaps twice that number of SNPs in commercial databases, such as that of Celera Genomics²⁰. The SNPs are now being verified experimentally, and their distributions between and within populations are being assessed²¹.

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www.nature.com/scientificgenetics

QIAGEN Exhibit 1012
Page 1

The next generation technology for SNP analysis should avoid the PCR amplification step, and the technology could be based on the analysis of single DNA molecules, which would allow direct determination of the haplotypes formed by the SNPs. Such

EX1012.0940

When using intercalating dyes or labelled allele-specific PCR primers without a consecutive target-specific detection reaction or size-separation step, the specificity of the method might be hampered owing to PRIMER DIMERS and other spurious amplification products that will not be distinguished from the actual PCR products.

EX1012.0935

IPR2025-00028: Paper 17, 35-41, 54-56; Paper 27, 16-18; IPR2025-00029: Paper 17, 35-41, 54-56; Paper 27, 16-18; EX2107.1170-173, 234-236, 252

Syvänen Praises Methods That Exclude PCR Amplification

REVIEWS

ACCESSING GENETIC VARIATION:
GENOTYPING SINGLE NUCLEOTIDE
POLYMORPHISMS

Table 2 | Features of SNP-genotyping methods

Method	Most significant advantage	Disadvantage	Special feature
Hybridization methods			
Reverse dot blot ²⁸	Widely used	Limited genotype discrimination	
GeneChip microarrays ³⁹	Multiplexing possible	Prone to non-specific background	Precursor to microarrays
DASH ⁴⁴	Very high probe density	High failure rates ^{41,42} , expensive	High-tech manufacturing process ⁴⁰
PNA ⁴⁶ and LNA ⁴⁸ probes	Inexpensive labelling method	Complex design rules	Dedicated instrument
TaqMan ^{49,50}	Specific as ASO probes	Not widely available	Innovative chemical design
Molecular Beacons ^{51,52}	Simplicity of assay	Expensive probes	Quantitative real-time PCR
Allele-specific PCR			
Intercalating dye ⁶⁰	Simplicity of assay	Expensive probes	Versatile stem-loop structure
FRET primers ^{61,62}	Real-time PCR assay	Requires optimization	
AlphaScreen ⁶⁴	Inexpensive labelling method	Non-specific products detected	Accurate quantification ¹³⁸
Primer extension			
SNPstream, GBA ⁶⁸	Simplicity of assay	Expensive primers	Universal FRET primers
Multiplex minisequencing,	Detects specific product	Expensive probes	Applicable to ASO hybridization
	Accurate genotyping	Similar reagents as in PCR	
	Inexpensive and robust	Multiple detection steps	Automated high-throughput process
	Multiplexing capacity	Size separation step	Compatible with capillary DNA

Ligase chain reaction¹⁰²

Alternative to PCR

Lower efficiency than PCR

Thermostable ligase

Padlock probes¹⁰⁸

Localized detection

Probes difficult to produce

Avoids target amplification

* * *

Invader assay¹¹²

PCR amplification avoided

Requires large amount of DNA

Interesting FLAP endonuclease

2005 Upgrade, Novartis
e-mail: Ann-Christina.
Syvanen@Novartis.com

Conclusion: Dr. Syvänen, Novartis, has received an
award for his research on the genetic and disease
causation of the phenotype of an individual.

Conclusion: The SNP is now being verified exper-
imentally, and their distribution between and within
populations are being assessed¹⁰¹.

*** JOURNAL OF THE

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QIAGEN Exhibit 1012
Page 1

Ligase chain reaction¹⁰²

Alternative to PCR

Lower efficiency than PCR

Thermostable ligase

Padlock probes¹⁰⁸

Localized detection

Probes difficult to produce

Avoids target amplification

Holling circle amplification⁹⁹

Signal amplification

Steric hindrance on solid phases

Based on circularized
oligonucleotides

Endonuclease cleavage

Restriction site analysis

Inexpensive, requires
no equipment

Not suitable for high throughput

Traditional method

Invader assay¹¹²

PCR amplification avoided

Requires large amount of DNA

Interesting FLAP endonuclease

APEX, arrayed primer extension; ASO, allele-specific oligonucleotide; DASH, dynamic allele-specific hybridization; ELISA, enzyme-linked immunosorbent assay; FRET, fluorescence resonance energy transfer; GBA, genetic bit analysis; LNA, locked nucleic acid; OLA, oligonucleotide ligation assay; PNA, peptide nucleic acid; TDI, template-directed incorporation.

EX1012.0934

IPR2025-00028: Paper 17, 36-37; Paper 27, 16-18

IPR2025-00029: Paper 17, 36-37; Paper 27, 16-18

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

EX2053.1008

Quail 2011

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

EX2071.0010

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

EX2054.0291

Petitioner's Expert Taught Away From Using PCR With NGS

Metzker

studies. The concept of targeting specific regions of the genome is well established, with PCR being the most widely used method, albeit on a small scale. PCR coupled with Sanger sequencing is suitably matched for analysing a handful of candidate genes⁶⁵, but coupling PCR with high-throughput NGS platforms for targeting strategies is not practical, as sample preparation would require handling tens of thousands of primers individually or in large multiplex groups to meet the needs of a single instrument run. A recent article by Frazer and colleagues

REVIEWS

APPLICATIONS OF NEXT GENERATION SEQUENCING

Sequencing technologies — the next generation

Michael L. Metzker^{1*}

Abstract | Demand has never been greater for revolutionary technologies that deliver fast, inexpensive and accurate genome information. This challenge has catalysed the development of next-generation sequencing (NGS) technologies. The inexpensive production of large volumes of sequence data is the primary advantage over conventional methods. Here, I present a technical review of template preparation, sequencing and imaging, genome alignment and assembly approaches, and recent advances in current and near-term commercially available NGS instruments. I also outline the broad range of applications for NGS technologies, in addition to providing guidelines for platform selection to address biological questions of interest.

Automated Sanger sequencing

The automated Sanger sequencing method, developed by Roberts and colleagues in 1986, has been the standard for sequencing DNA for over two decades. It is a highly accurate and reliable method for sequencing DNA, but it is also a labor-intensive and expensive process. The development of NGS technologies has led to a shift away from the application of the Sanger method for genome analysis. Prior to this departure, the automated Sanger method had dominated the industry for almost two decades and led to a number of monumental accomplishments, including the completion of the first human genome sequence and the sequencing of the genomes of many other organisms. Despite some technical improvements during this time, the limitations of automated Sanger sequencing showed a need for new and improved technologies for sequencing large numbers of human genomes. Recent efforts have been directed towards the development of new methods, leaving Sanger sequencing with lower reported advances. As such, automated Sanger sequencing is not

Over the past four years, there has been a fundamental shift away from the application of the Sanger method for genome analysis. Prior to this departure, the automated Sanger method had dominated the industry for almost two decades and led to a number of monumental accomplishments, including the completion of the first human genome sequence and the sequencing of the genomes of many other organisms. Despite some technical improvements during this time, the limitations of automated Sanger sequencing showed a need for new and improved technologies for sequencing large numbers of human genomes. Recent efforts have been directed towards the development of new methods, leaving Sanger sequencing with lower reported advances. As such, automated Sanger sequencing is not

determining the order of bases. For example, in gene-expression studies microarrays are now being replaced by next-generation sequencing, which can identify and quantify gene transcripts without prior knowledge of a particular gene and can provide information regarding alternative splicing and sequence variation in identified genes⁶⁶. The ability to sequence the whole genomes of many related organisms has allowed large-scale comparative and evolutionary studies to be performed that were unimaginable just a few years ago. The broadest application of NGS may be the sequencing of human genomes to enhance our understanding of how genetic differences affect health and disease. The variety of NGS features makes it likely that multiple platforms will continue to be developed to address various biological

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NATURE REVIEWS | GENOMICS

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QIAGEN Exhibit 1025
Page 1

EX1025.0041

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



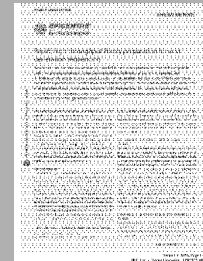
Ernani,
EX2009.0001
(August 2009)

“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.**”



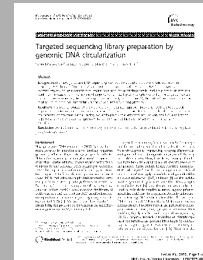
Moorthie,
EX2169.0007
(October 2011)

“**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,
EX2056.0003
(August 2010)

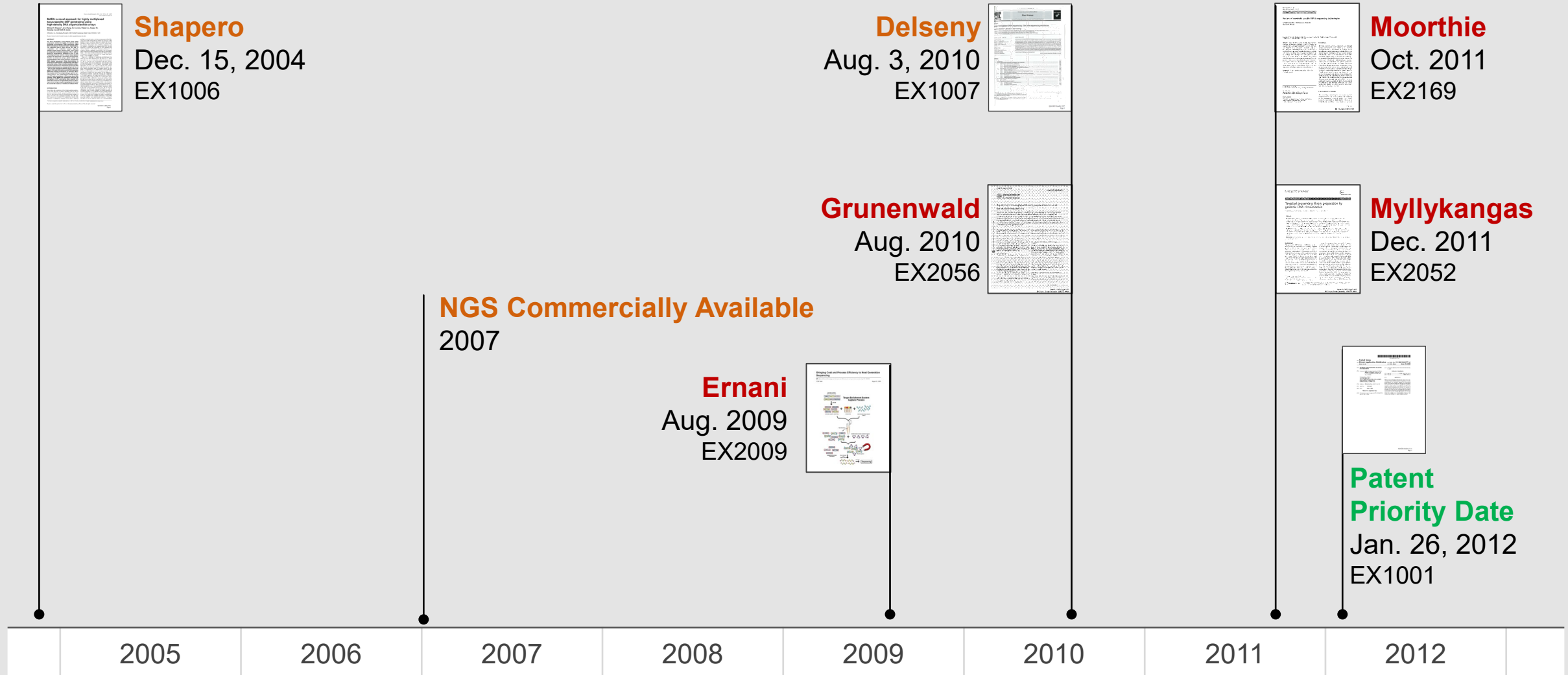
“Current methods (both mechanical and enzymatic) face limitations: **multi-step protocols, sample loss, lack of automation and labor costs**. With the continued decline of sequencing costs and increase in sample throughput, there is **greater demand for more cost effective, streamlined library preparation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

Long-Felt, Unmet Needs Persisted After Shapero



IPR2025-00028: Paper 17, 57-59; Paper 27, 25; IPR2025-00029: Paper 17, 57-59; Paper 27, 25; EX2107. ¶¶288-292

Industry Praised Tecan's SPET Inventions

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,

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.

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

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.

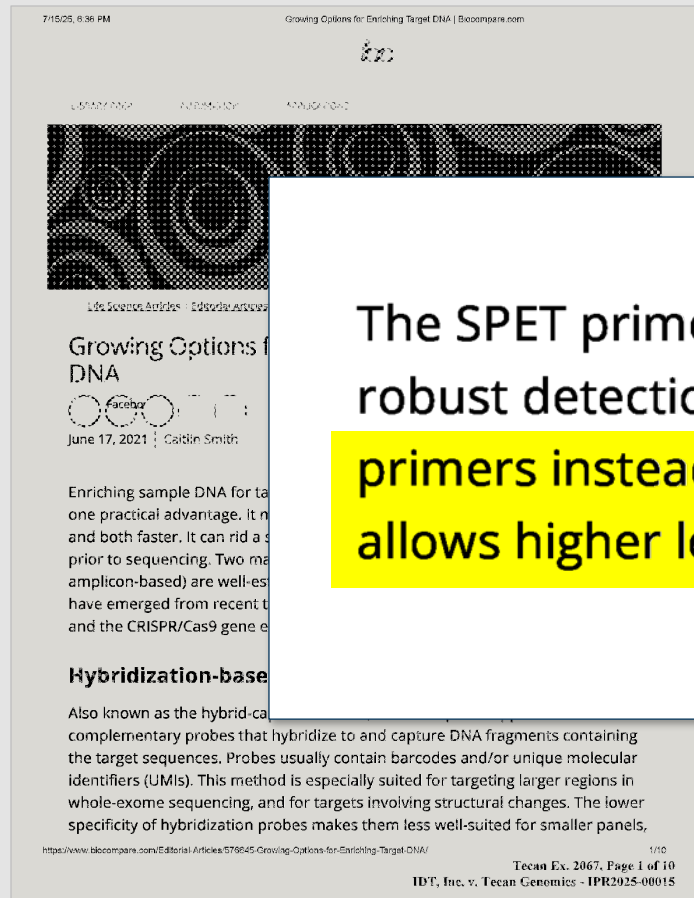
EX2065.0001-02, 12-13

Industry Praised Tecan's SPET Inventions

Smith

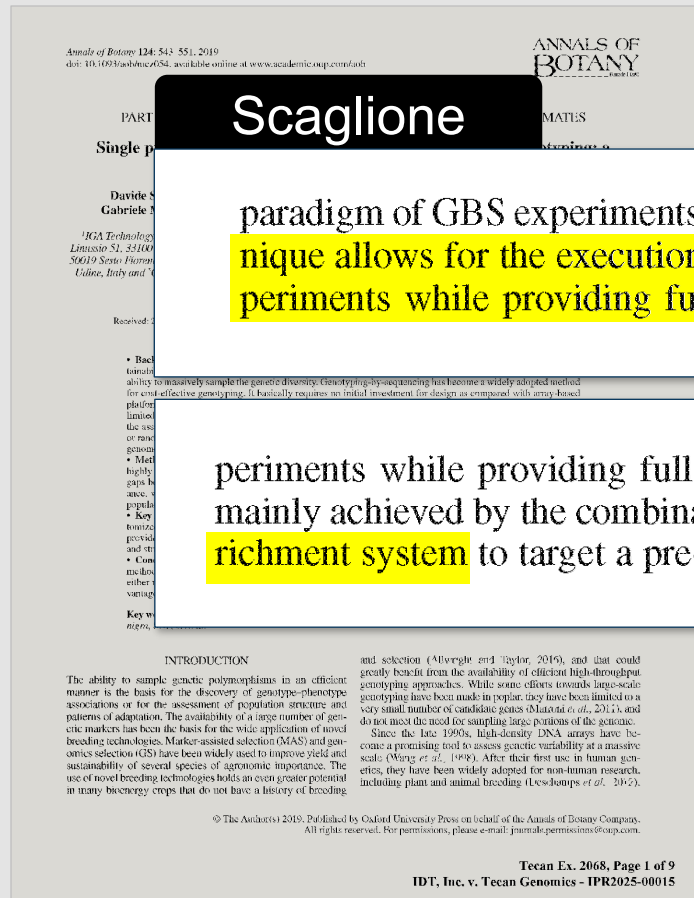
The SPET primers are targeted to both strands of the target sequence for more robust detection in SNP analysis, CNV analysis, and re-sequencing. Using single primers instead of primer pairs greatly simplifies the design of primer panels, and allows higher levels of multiplexing compared to amplicon-based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

EX2067.0003



IPR2025-00028: Paper 17, 59-61; Paper 27, 25-26; IPR2025-00029: Paper 17, 59-61; Paper 27, 25-26; EX2107.11293-298

Industry Praised Tecan's SPET Inventions



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

EX2068.0548

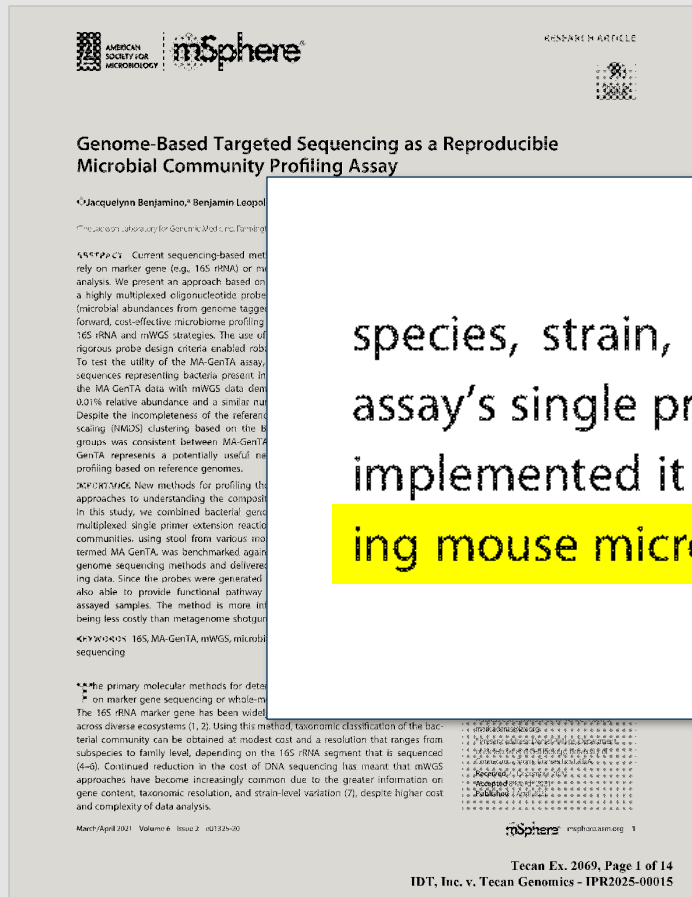
in every locus. Our results suggest that SPET genotyping is a very effective approach for genotyping a large number of loci, and we suggest that it might be a valuable tool for large-scale genotyping studies in virtually all species. This will greatly facilitate molecular breeding approaches for bioenergy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

EX2068.0550

IPR2025-00028: Paper 17, 59-61; Paper 27, 25-26; IPR2025-00029: Paper 17, 59-61; Paper 27, 25-26; EX2107.¶¶293-298

Industry Praised Tecan's SPET Inventions

Benjamino

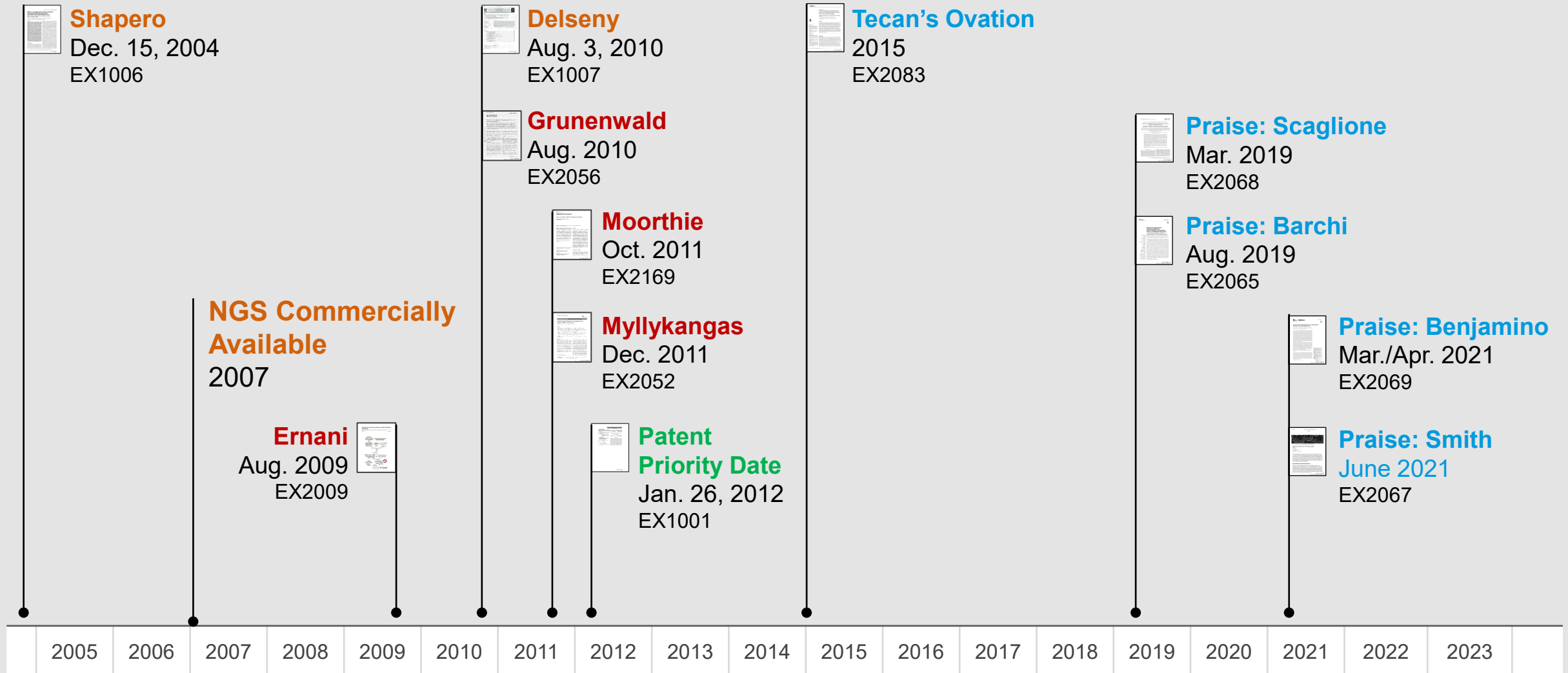


species, strain, and even gene level. We adapted the Allegro Targeted Genotyping assay's single primer extension reaction that is widely used for genotyping (17, 18) and implemented it as a quantitative, straightforward, and cost-effective method for profiling mouse microbial communities based on the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

EX2069.0002

IPR2025-00028: Paper 17, 59-61; Paper 27, 25-26; IPR2025-00029: Paper 17, 59-61; Paper 27, 25-26; EX2107.¶¶293-298

No Praise for Shapero's Library Preparation Method



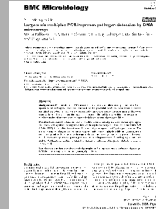
IPR2025-00028: Paper 17, 45-47, 59-61; Paper 27, 25-26; IPR2025-00029: Paper 17, 45-47, 59-61; Paper 27, 25-26; EX2107. ¶¶272-274, 293-298

Shapero Did Not Solve the Long-Felt, Unmet Needs



Bui,
EX2138.0002
(January 2009)

“impractical for assaying a selected set of SNPs in specific genomic regions, and [is] usually beyond the reach of small to medium sized laboratories with limited resources.”



Palka-Santini,
EX2139.0010
(January 2009)

“involve[d] multiple steps starting with enzymatic digestion of the template DNA, ligation to adapters, primer extension and finally two subsequent PCR reactions. Such multi-step approaches are **time consuming and prone to contamination** and therefore have not been recommended for bacteriological routine diagnostics.”



Liu,
EX2141.0002
(August 2012)

“expensive and require specialized equipments, which cost more [than] standard primers and are not practical for assaying low- to moderate-throughput SNPs. ... there is a **need for simple and accurate genotyping assays that can be implemented in laboratories lacking access to sophisticated equipment.**”

Secondary Considerations Relevant When Combination Is Novel



The Board acknowledged Medtronic showed every element of the Side Opening Claims was *individually* known in the prior art but correctly concluded this did not preclude nexus where the evidence was “tied to the combination of features as a whole” and the combination was not previously known.

Medtronic, Inc. v. Teleflex Innovations S.a.r.l., 70 F.4th 1331, 1337 (Fed. Cir. 2023) (emphasis original)



**Carlos Bustamante,
Ph.D.**

276. As explained above, the claimed inventions are directed to the use of a single target-specific primer (the “one or more oligonucleotides” with “at least 10 bases designed to be complementary to the nucleic acid sequence of interest”) to selectively amplify and enrich a target sequence with a pair of common adapters that can then be sequenced on a massively parallel sequencing platform. *See supra* ¶¶ 118-121. This combination of limitations is unique and characteristic for all claims of both patents. Tecan (and its predecessor, NuGEN) refer to the claimed technology as “Single-Primer Enrichment Technology” or “SPET.”

EX2107.¶276

Benefits of Invention Need Not Be Claimed



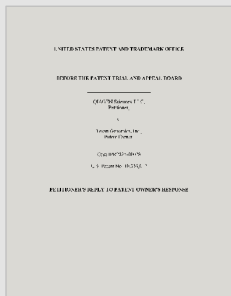
We have already rejected Kohler's argument that the objective considerations lack nexus because they are not tied specifically to the coolant elements of the asserted claims.

WBIP, LLC v. Kohler Co., 829 F.3d 1317, 1332 (Fed. Cir. 2016)

Petitioner Misconstrues the Art Discussing Shapero

EX2139, published years before Shapero, could not be commenting on his results. EX2130 is only stating that Shapero does not produce zero undesired

Paper 22, 20



Shapero

Accepted November 24, 2004

EX1006.0001



Palka-Santini

Published: 3 January 2009

EX2139.0001

Petitioner Misapplies the Law on Copying



Evidence of access and substantial similarity is evidence of copying. Medtronic's argument is tantamount to requiring that copying can be proven only by direct evidence “such as photos of patented features or disassembly of products,” *Liqwd*, 941 F.3d at 1137, rather than circumstantial evidence such as access and similarity. Our case law has never drawn any such distinction, nor would such a distinction be consistent with basic principles of evidence and inference. **Circumstantial evidence is just that—evidence. It establishes that a given fact is more likely to be true than one would otherwise believe in the absence of the evidence.** Nor is circumstantial evidence second-class to direct evidence: “Circumstantial evidence is not only sufficient, but may also be more certain, satisfying and persuasive than direct evidence.” *Michalic v. Cleveland Tankers, Inc.*, 364 U.S. 325, 330, 81 S.Ct. 6, 5 L.Ed.2d 20 (1960) (citation omitted).

Medtronic, Inc. v. Teleflex Innovations S.a.r.l., 70 F.4th 1331, 1340 (Fed. Cir. 2023) (emphases modified)

QIAGEN Had Access to Claimed Technology

Case 1:23-cv-01115-GBW Document 11 Filed 01/05/24 Page 1 of 88 PageID #: 390

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

TECAN GENOMICS, INC.,)
Plaintiff,) C.A. No. 23-1115-GBW
v.) JURY TRIAL DEMANDED
QIAGEN SCIENCES, LLC, QIAGEN)
GATTHERSBURG, LLC f/k/a QIAGEN)
GATTHERSBURG, INC., QIAGEN GMBH,)
and QIAGEN N.V.,)
Defendants.)

DEFENDANTS' ANSWER AND DEFENSES

Defendants QIAGEN Sciences, LLC, QIAGEN Gaithersburg, LLC, QIAGEN GmbH, and QIAGEN N.V. (collectively "QIAGEN"), by and through the undersigned attorneys, hereby provides the following answer and defenses in response to the Complaint of Tecan Genomics, Inc. ("Tecan"). To the extent not specifically admitted herein, the allegations of the Complaint are denied. QIAGEN denies any allegations that may be implied by or inferred from the headings of the Complaint.

ANSWER

Nature of the Action

1. Tecan Genomics brings this action to halt Defendants' infringement of, and seek damages for, Defendants' past, ongoing, and future acts of infringement of U.S. Patent Nos. 10,036,012 ("the '012 patent"), 10,876,108 ("the '108 patent"), 11,098,357 ("the '357 patent"), and 11,725,241 ("the '241 patent") (collectively "the Asserted Patents") (attached hereto as Exhibits 1-4 respectively).

RESPONSE: QIAGEN admits that Tecan's Complaint purports to allege claims for past, ongoing, and future acts of infringement of U.S. Patent Nos. 10,036,012 ("the '012 patent"), 10,876,108 ("the '108 patent"), 11,098,357 ("the '357 patent"), and 11,725,241 ("the '241 patent")

Tecan Ex. 2170, Page 1 of 88
QIAGEN Sciences, LLC v. Tecan Genomics - IPR2025-00028

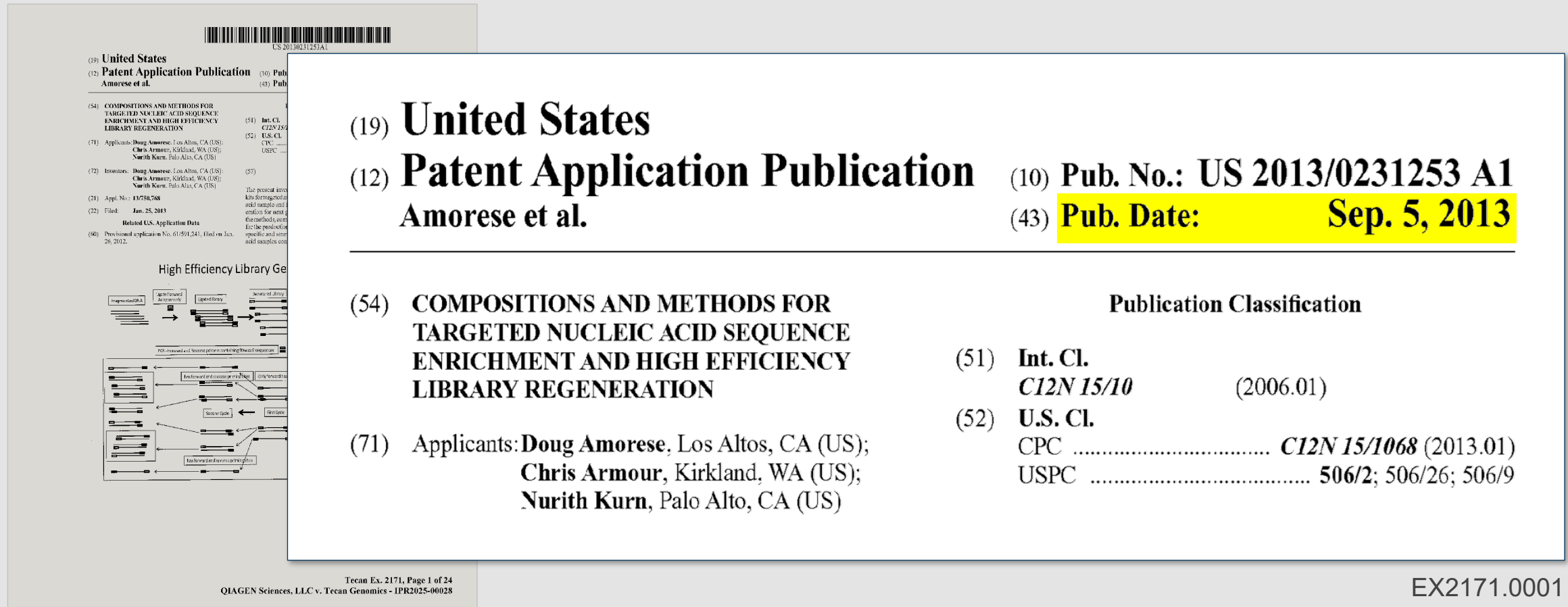
RESPONSE: QIAGEN admits that NuGEN and QIAGEN entered into discussions related to QIAGEN's potential acquisition of NuGEN. QIAGEN denies that the discussions between NuGEN related to QIAGEN's potential acquisition of NuGEN began in December 2013. QIAGEN admits that it performed some level of due diligence regarding acquisition of NuGEN. QIAGEN admits that QIAGEN's due diligence involved discussions with QIAGEN employees and reviewing various documents. QIAGEN denies that it received unfettered access to

* * *

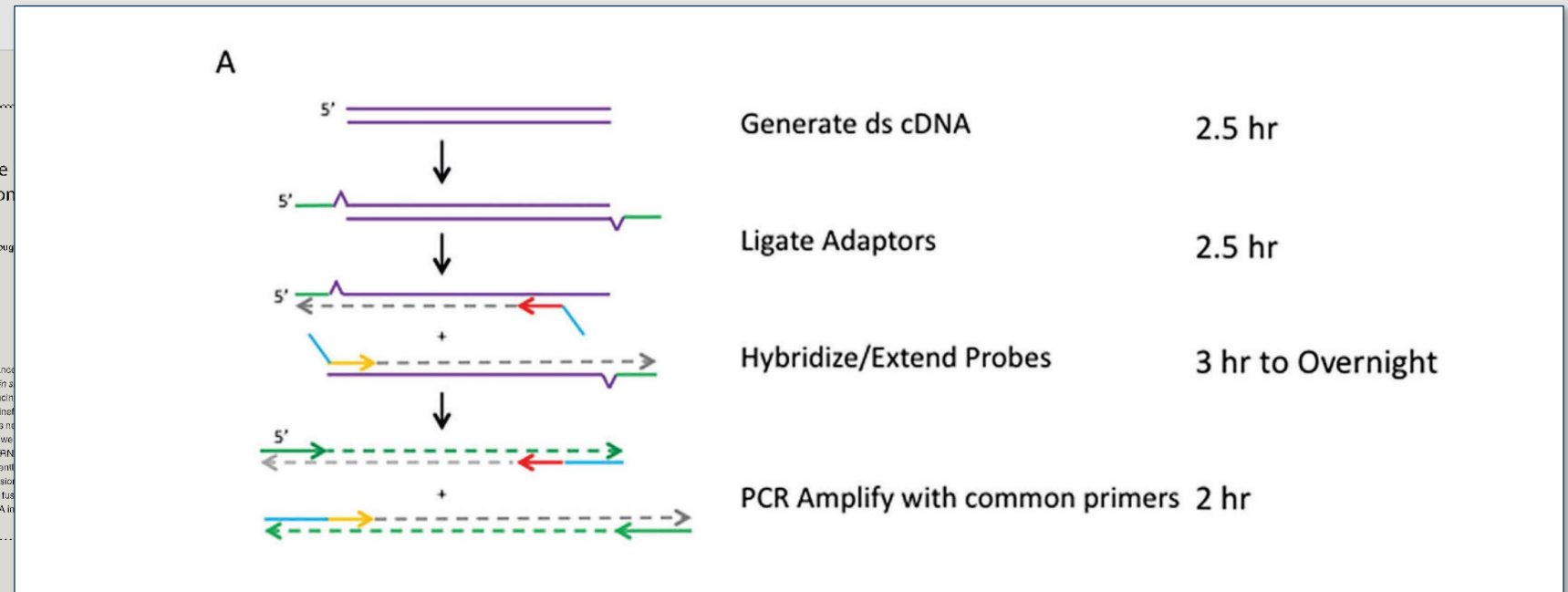
RESPONSE: QIAGEN admits that it received certain detailed information on NuGEN technologies. But at this time QIAGEN is unable to determine if QIAGEN requested from NuGEN

EX2170.0031-32 (Petitioner's Responses to Paragraphs 70-71)

Tecan Published the Patented Workflow in 2013



Tecan Published Its Ovation Workflow in 2015



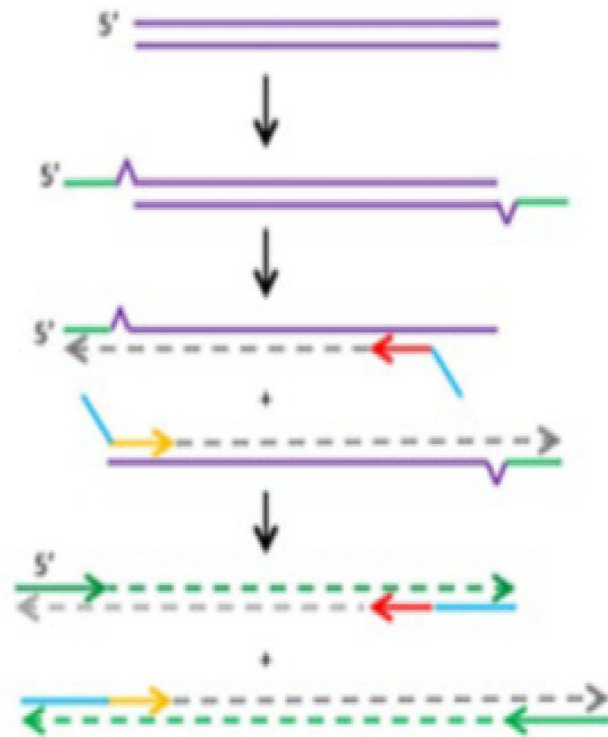
The resulting library was sequenced on an Illumina MiSeq

EX2083.0002-03

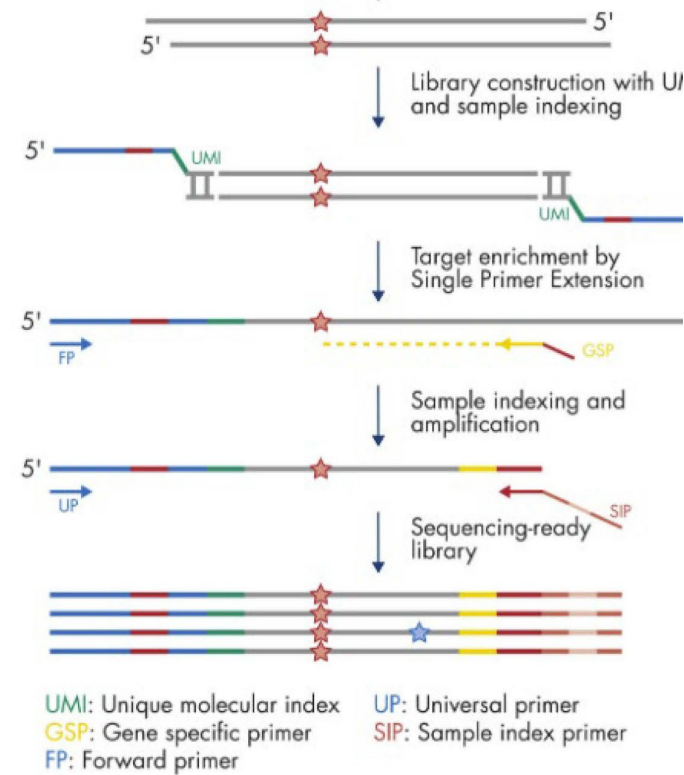
IPR2025-00028: Paper 17, 62-64; Paper 27, 26-27; IPR2025-00029: Paper 17, 62-64; Paper 27, 26-27; EX2107. ¶¶299-304

QIAGEN's Later Workflow Is Substantially the Same As Tecan's Ovation Workflow

Ovation Target Enrichment



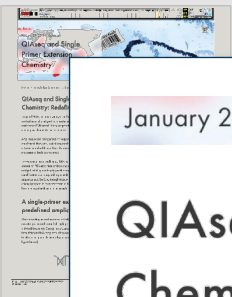
QIAseq Targeted DNA Panel



EX2107.¶¶301-303

IPR2025-00028: Paper 17, 62-64, Paper 27, 26-27; IPR2025-00029: Paper 17, 62-64, Paper 27, 26-27; EX2107.¶¶299-304

QIAGEN Marketed Its Copied Products As “Revolutionary”



January 29, 2018 | Genomics

QIAseq and Single Primer Extension Chemistry: Redefining Amplicon-Based NGS

Compared to existing targeted enrichment approaches, the SPE method relies on single end adapter ligation, which inherently has a much higher efficiency than requiring adapters to ligate to both ends of the dsDNA fragment. More DNA molecules will be available for the downstream PCR enrichment step.

EX2147.0005 (January 2018)

EX2148.0002 (February 2018)

QIAseq™ SPE technology for Illumina®: Redefining amplicon sequencing

Single primer extension (SPE) is a revolutionary approach that redefines amplicon-based enrichment and sequencing. With SPE, each genomic region is targeted by only one region-specific primer plus a universal adapter primer that binds to sequences introduced through library adapters (Figure 4).

QIAseq SPE technology for Illumina: Redefining amplicon sequencing 02/2018



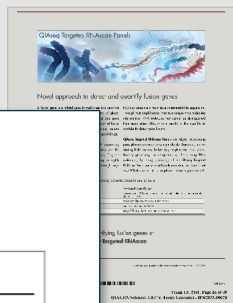
EX2146.0006 (August 2016)

Novel approach to detect and quantify fusion genes

QIAseq Targeted RNAscan Panel (cat. nos. 333602, 333605, 333625 and 333645)

What's new?	How does this benefit you?
Molecular barcoding of transcripts	Does not count PCR duplicates, accurate quantification of fusion transcripts, higher sensitivity
Single primer extension	Detect both known and novel fusion genes
Single pool of primers	Requires low amount of RNA
Short library fragments	Suitable for low yield, poor quality RNA samples

Exploring new frontiers with next-generation sequencing 08/2016



IPR2025-00028: Paper 17, 64-67, Paper 27, 27; IPR2025-00029: Paper 17, 64-67, Paper 27, 27; EX2107.11305-309

QIAGEN Repeatedly Extolled Using a Single Primer

However, current amplicon-based enrichment and sequencing methods that rely on either two-primer designs or nested PCR approaches suffer from the following inefficiencies:

- Low uniformity and lack of coverage due to primer dropouts
- Requirement for multiple primer pools resulting in increased DNA input per sample (see Figure 1)
- Need of a large contiguous stretch of the targeted regions to cover two primers for a nested PCR design (see Figure 2)
- Inflexibility with regards to adding primers to increase panel content
- Low library complexity as both start and stop positions are defined by primers (see Figure 3)
- Inefficiency to cover entire CDS regions

Single primer extension (SPE) is a revolutionary approach that redefines amplicon-based enrichment and sequencing. With SPE, each genomic region is targeted by only one region-specific primer plus a universal adapter primer that binds to sequences introduced through library adapters (Figure 4).

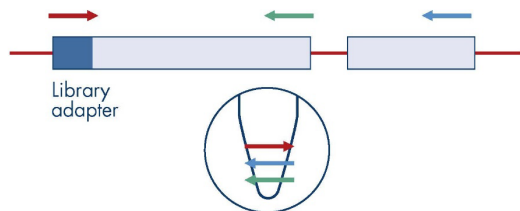
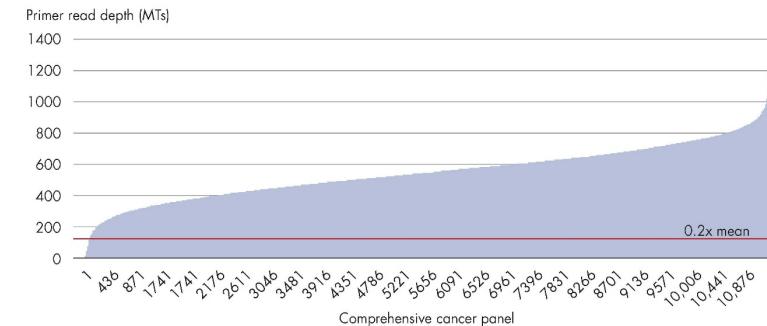
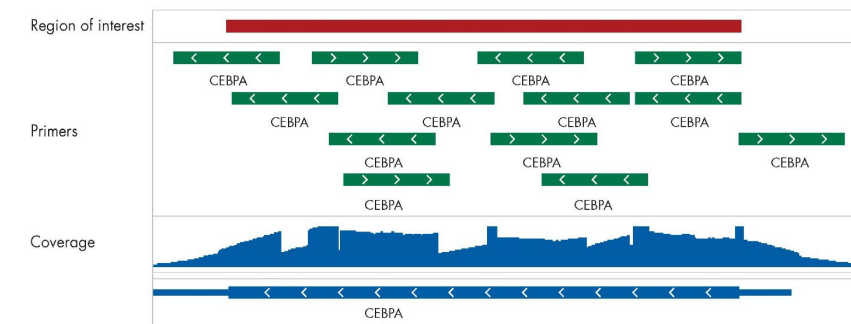


Figure 4. Primer design using single primer extension (SPE). Amplicon-based enrichment is performed in a single reaction regardless of the size of targeted regions.

SPE utilizes a reduced number of required primers resulting in highly uniform enrichment and sequencing due to a reduction in primer dropouts (Figure 5).



As opposed to traditional two-primer amplicon and nested PCR approaches, SPE provides a high degree of primer design flexibility, allowing for primers to be aggressively tiled across targeted regions (Figure 6).

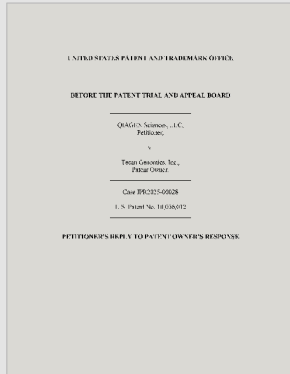


EX2148.0001-02

IPR2025-00028: Paper 17, 64-67, Paper 27, 27; IPR2025-00029: Paper 17, 64-67, Paper 27, 27; EX2107.¶¶305-309

EX2148.0002-03

QIAGEN's Novel Copying Defense



**Michael Metzker,
Ph.D.
Petitioner's Expert**

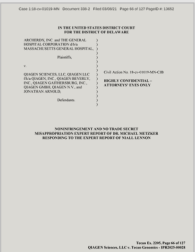
Last, PO points to a side-by-side comparison of the library preparation steps in PO's product and QIAGEN's products and claims similarities establish copying. POR.63-64. Dr. Bustamante admitted, however, the QIAGEN workflow is closer to the Shapero workflow than PO's. EX1120.390:2-402:9.

- Q. Okay. Do you agree that the Qiagen workflow is closer to the Shapero workflow than patent owners?
- A. Well, I agree -- again, you pointed out that I was present during Dr. Bustamante's deposition when these questions were asked, and I do agree with this -- with the steps that were shown to be side by side that the Shapero workflow was closer to the Qiagen workflow than the Tecan workflow.

Paper 22, 24

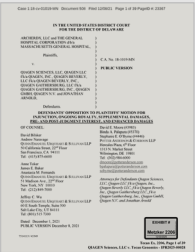
EX2213.0069:18-70:5

QIAGEN Argued the Opposite to the Federal Courts



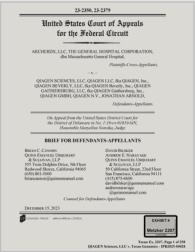
- A. The Accused QIAGEN Products Use Only One PCR Step With a Gene Specific Primer Followed by One Universal PCR Step..... 29
- B. Inventors of the '810 Patent and '597 Patent Were Surprised That QIAGEN Could Achieve High Specificity Without a Second PCR Round Using Nested Gene-Specific Primers 32

EX2205.0068



comparison of results. Third, Plaintiffs’ patents teach away from the methods QIAGEN developed. In the vernacular of the trial, Archer’s products use two panning-for-gold steps (Tr. 201:12-203:2 282:2-283:4), while QIAGEN’s use only one (Tr. 519:23-520:4, 525:24-526:5, 527:14-25, 528:18-529:13; Tr. 908:6-25). QIAGEN did what Archer’s inventors said it was unable to do: produce a product with only one enrichment step. Tr. 214:5-18, 215:12-18; Tr. 278:15-279:24; DX-44; DX-116. Archer’s products contain nested adaptor and target specific

EX2206.0026



This includes the UP, FP and SIP. *Id.* QIAGEN carefully designed its process and constructed its GSPs so that its process can enrich for the sequence to be analyzed with a single pair of primers: the GSP and the FP.

EX2207.0011

QIAGEN Represented the Opposite to the Patent Offices

Munich, 26 October 2020

1. Clarity (Art. 84 EPC)

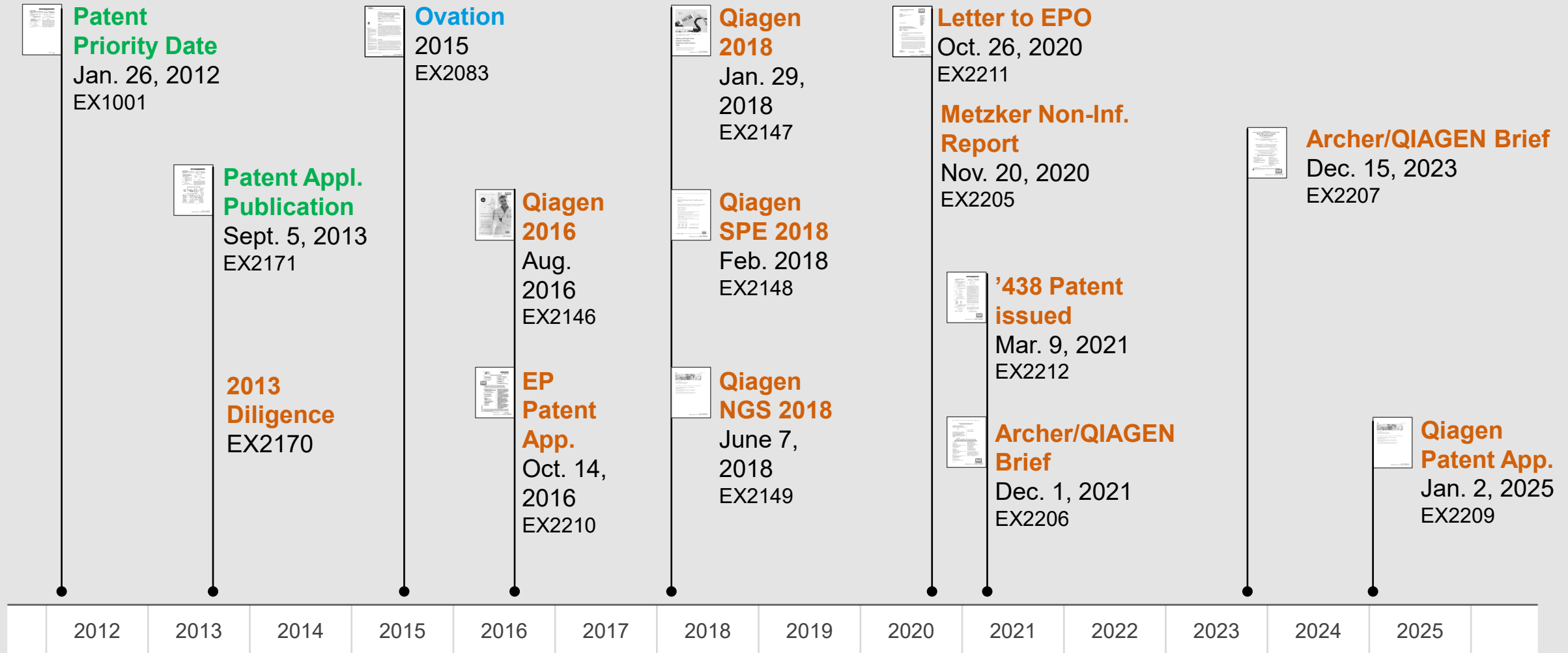
- 1.1 In the IPRP, item VIII, claim 1 is considered to lack clarity as the Examiner considers that there is no disclosure in the description as to how a multiplex single primer extension reaction could be performed with 1000 different primers. It seems there is some confusion surrounding the combination of “single primer” and “1000 different primers.” This relies on a misunderstanding with respect to the primers used in claim 1. There are 1000 different target specific primers, however each primer only binds to one target and these primers are not complimentary. The one primer bound to one target is then extended to amplify the target sequence. There is no reverse primer directed to the other end of the DNA, the amplification only takes place from one end. **This difference is crucial, because it is what differentiates the claimed methods from the standard prior art methods for amplifying polynucleotides, which require a forward and a reverse primer.**

EX2211.0001-02

IPR2025-00028: Paper 27, 26-27; Paper 33, 1-7; IPR2025-00029: Paper 27, 26-27; Paper 33, 1-7

Demonstrative Exhibit - Not Evidence

QIAGEN Consistently Praised the Copied Products



IPR2025-00028: Paper 27, 26-27; Paper 33, 1-7; IPR2025-00029: Paper 27, 26-27; Paper 33, 1-7

QIAGEN Published Unexpected Results of SPE

June 7, 2018 | Genomics

Issues with primer dropouts

June 7, 2018 | Genomics

Issues with primer dropouts

Home > Knowledge & Support > Knowledge Hub > Science Matters > Genomics > Having issues with primer dropouts

Having issues with primer dropouts in your 2-primer amplicon-based panel? You should!

Don't you hate seeing coverage gaps in your NGS panel after you've run your primers correctly? Especially when the reason for the gap is as simple as primer dropout? What's worse is that filling this gap might require a complete re-design of your panel.

Well, this is not an uncommon problem for panels that utilize a 2-primer amplicon approach for sequencing. Examples of how coverage due to primer dropouts may be seen in the paper, and the table below as a summary.

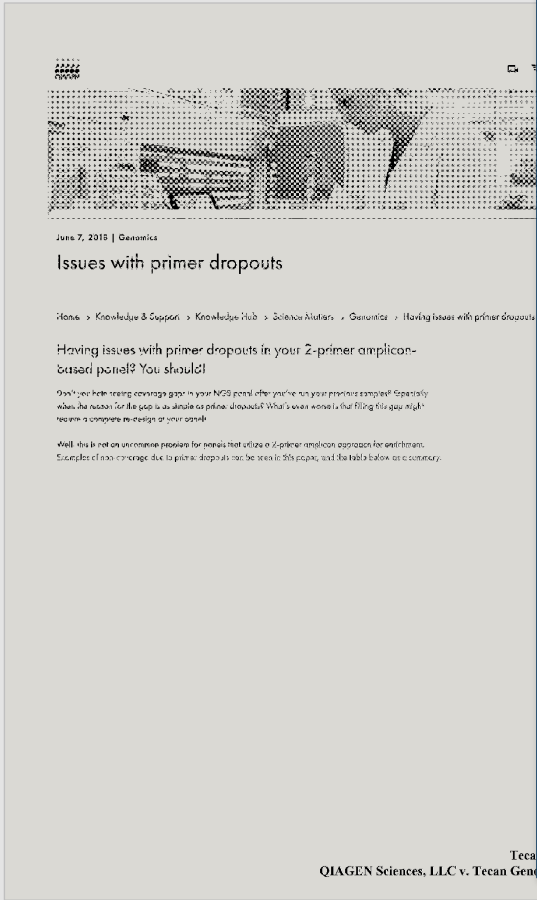
Tecan Ex. 2149, Page 1
QIAGEN Sciences, LLC v. Tecan Genomics - IPR2025-00028

EX2149.0005

Gene	2-primer amplicon		SPE in QIAseq		
	Region	Coverage	Region	Coverage	LOD
<i>Akt1</i>	Chr14: 105246446–105246583	0	Chr14: 105246245–105246553 NM_001014431	5734	0.4%
<i>FGFR3</i>	Chr4: 1806082–1806187	0	Chr4: 1806057–1806247 NM_000142	4406	0.5%
<i>NOTCH1</i>	Chr9: 139399338–139399447	0	Chr9: 139399125–139399556 NM_017617	5406	0.4%
<i>STK11</i>	Chr19: 1220481–1220603	0	Chr19: 1220372–1220504 NM_000455	5286	0.4%
<i>TP53</i>	Chr17: 7579351–7579485	0	Chr17: 7579312–7579590 NM_001276761	5740	0.4%
	Primer dropout		No primer dropout		

IPR2025-00028: Paper 17, 67-69; Paper 27, 27-28; IPR2025-00029: Paper 17, 67-69; Paper 27, 27-28; EX2107.¶¶310-319

QIAGEN Acknowledged the Unexpected, Superior Results of SPE



2-primer amplicon design



Two primers are required to define a targeted region

Outcomes:

- Increased risk of primer dimers and dropouts
- Lower uniformity
- Multiple pools of primers might be required
- Low library complexity
- Increasing panel content might require primer redesign

SPE amplicon design – QIAseq



Only one primer is required to define a targeted region

Outcomes:

- Lower risk of primer dimers and dropouts
- Higher uniformity
- Only a single pool of primers required
- Higher library complexity
- Increasing panel content does not require primer redesign

EX2149.0003-04



ORAL ARGUMENT FOR IPR2025-00028 IPR2025-00029

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

Patent Owner's Hearing Demonstratives

February 11, 2026

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the foregoing **PATENT OWNER'S HEARING DEMONSTRATIVES** was served on February 6, 2026 and February 9, 2026 by email to Petitioner by serving the below counsel of record:

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