

Jim

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

This opinion in support of the decision being entered today (1) was not written for publication in a law journal and (2) is not binding precedent of the Board.

Paper No. 50

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte R. BRUCE WALLACE

Appeal No. 93-2889
Application 07/071,210¹

HEARD: MAY 8, 1995

MAILED

AUG 14 1995

PAT. & TM. OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

Before MCKELVEY, Chief Administrative Patent Judge, and
WILLIAM F. SMITH and GRON, Administrative Patent Judges.

GRON, Administrative Patent Judge.

DECISION ON APPEAL

1. Introduction

This is an appeal under 35 U.S.C. § 134 from an examiner's final rejection of Claims 6, 7, 9 and 10, all claims pending in this application, under 35 U.S.C. § 103 over the combined teachings of R. Bruce Wallace, et al. (Wallace), Nucleic Acids Research, Vol. 9, No. 4, pages 879-894 (1981) and Saiki, et al. (Saiki), U.S. Patent No. 4,683,194, filed March 28, 1985, issued

¹ Application for patent filed July 8, 1987.

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July 28, 1987. The claims stand or fall together (Appellant's Brief, page 9 (Br9)). Representative Claim 6 is reproduced in the attached appendix.

2. The invention claimed

The claims are directed to a hybridization assay for target RNA (RNA_1) in a biological sample containing a mixture of the RNA_1 and an RNA_2 which differs from RNA_1 by one nucleotide. The method comprises adding to the sample a mixture of oligonucleotide probes including a first labeled probe (P_1^*) which is complementary to (matches) and hybridizes with RNA_1 in the presence of a substantial excess of a second probe (P_2) which is not complementary to (mismatches) RNA_1 , is complementary to (matches) RNA_2 , and suppresses hybridization of P_1^* with RNA_2 .

The claimed hybridization assay can be illustrated as follows:

$RNA_1 + RNA_2 + P_1^* + \text{excess } P_2 = \text{match } RNA_1 \parallel P_1^* + \text{match } RNA_2 \parallel P_2 + P_2.$

The problem appellant's assay is designed to eliminate is interfering cross-hybridization of P_1^* with RNA_2 , i.e., the mismatch of RNA_2 with P_1^* ($RNA_2 \parallel P_1^*$) in conventional hybridization assays for RNA_1 using the labeled complementary probe P_1^* . The problem is illustrated below:

$RNA_1 + RNA_2 + P_1^* = \text{match } RNA_1 \parallel P_1^* + \text{mismatch } RNA_2 \parallel P_1^*.$

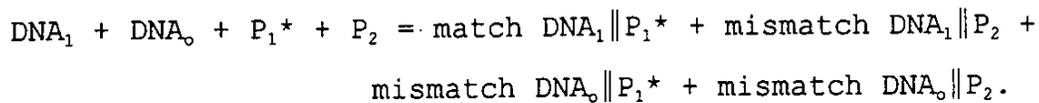
3. The prior art

A. Wallace

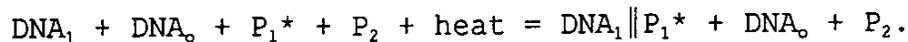
Wallace seeks to maximize hybridization of target DNA_1 with

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a probe P_1^* complementary to (matches) DNA_1 in the presence of other DNA (DNA_0) when probing with a combination of probes which differ in one nucleotide, i.e., P_1^* and P_2 . Wallace selectively identifies target DNA_1 by use of hybridization conditions which either prevent or eliminate mismatches. The mismatches persons having ordinary skill in the art reasonably would have expected Wallace to face can be illustrated as follows:



Wallace solved his mismatch problems by altering the hybridization conditions, i.e., the sample mixture of DNA is assayed by the mixed probes under stringent conditions, e.g., elevated temperatures at which perfect matches are stable and mismatches are not. The procedure is illustrated below:



According to appellant, Wallace never confronted the mismatch problems with which appellant's invention deals because Wallace's non-complementary probe P_2 would not and could not hybridize to either target DNA_1 or other DNA_0 under the stringent temperatures applied (Reply To Answer, Attachment A, pages 2-3, bridging ¶, and page 3, first full ¶):

It certainly would not have been obvious to one of ordinary skill in the art to add an excess of a probe which is capable of hybridizing to the MUT target sequence . . . to improve hybridization specificity. In fact, the Wallace reference suggests that adding an

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excess of a probe capable of hybridizing . . . would suppress specific hybridization (see page 888, Figure 3B lane d). In other words, one would have expected the presence of the excess of such an oligonucleotide to suppress both specific and non-specific hybridization and not to have increased specificity.

When a probe complementary to a target sequence does not hybridize to a different sequence with at least one non-complementary base, competition hybridization is not necessary to discriminate the two sequences (see Nozari et al, page 27 Figure 2A). This is not the present invention. When a probe complementary to a target sequence does hybridize to a different sequence with at least one non-complementary base, competitor is useful for the discrimination of the two sequences and is used (see Nozari, et al., page 27 Figure 2B and 2C and Wu et al., page 139, Figure 2). This is the present invention.

However, we agree with the examiner that appellant may be reading Wallace's disclosure much too narrowly. For example, Wallace states (Wallace, pages 888-891):

In order to test the use of the mixed probe in colony screening, transformed cells which contained either pBR322 or pBR322 β -globin were grown Ten of fifty colonies contained globin DNA sequences. . . . As expected, [³²P]R β G14A clearly hybridizes to the ten globin DNA containing colonies and not to the others. [³²P]R β G14B is not seen to hybridize in this exposure, but . . . exposed. . . longer, R β G14B also hybridizes to the ten globin DNA colonies. [³²P]R β G13Mix is seen to hybridize specifically with the globin DNA colonies, albeit with a significantly higher background due to the fact that eight-fold more labeled probe was present during the hybridization

Wallace nevertheless expressly states (Wallace, pages 887-888):

The ultimate application of oligonucleotides of mixed sequences would be to use them as probes to screen recombinant clones for those which contain the desired sequence.

Thus, we generally agree with appellant that Wallace endeavored

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to eliminate competitive hybridization. As a result, Wallace was not specifically concerned with mismatch problems prevalent in competitive hybridization assays.²

B. Saiki

We agree with the examiner that Saiki utilized a blocking oligomer to prevent non-specific binding of the target-complementary probe in hybridization assays for target DNA or RNA. However, Saiki's teaching of the use of a blocking agent is not the general proposition the examiner portrays. Rather, we agree with appellant (Reply To Answer, Attachment A, page 3, Saiki reference):

The Saiki reference does not describe a method to improve the specificity of a hybridization assay for discrimination of two sequences which differ by one or more nucleotides. This references [sic] merely utilizes a blocking oligonucleotide to reduce the background

² Conner, Brenda J., et al. (Conner), "Detection of sickle cell β^s -globin allele by hybridization with synthetic oligonucleotides," Proc. Natl. Acad. Sci., Vol.80, pages 278-282 (January 1983), appears even more pertinent to appellant's claimed invention than Wallace. However, we note that Conner, like Wallace, describes assays for target DNA using mixed probes, not assays for target RNA. Nozari, G., et al., "Discrimination among the transcripts of the allelic human β -globin genes β^A , β^S and β^C using oligo-deoxynucleotide hybridization probes," Gene, Vol. 43, pages 23-28 (1986) (Introduction, pages 23-24; not prior art), suggests that RNA mismatches were known to be far more stable than DNA mismatches at the time appellant's invention was made and therefore are much more difficult to eliminate by application of stringent hybridization conditions.

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. . . Saiki does not utilize competition hybridization at all. In Saiki, the blocking oligomer is complementary to the probe, while in the present invention the competitor oligonucleotide is complementary to the target; in Saiki the blocking oligonucleotide is added after the hybridization, while in the present invention the competitor oligonucleotide is present during the hybridization and finally, the purpose of the blocking oligonucleotide in Saiki is to hybridize to the probe that has not hybridized to the target, while the purpose of the competitor in the present invention is to hybridize to the non-target sequences such that the probe will not hybridize non-specifically.

4. Discussion

Saiki's two-stage assay in which DNA and RNA appear to be interchangeable (col.6, lines 12-25) can be illustrated as follows:

- (1) $DNA_1 + DNA_0 + P_1^* = \text{match } DNA_1 \parallel P_1^* + \text{mismatch } DNA_0 \parallel P_1^* + P_1^*$;
- (2) $\text{match } DNA_1 \parallel P_1^* + \text{mismatch } DNA_0 \parallel P_1^* + P_1^* + P_2 =$
 $\text{match } DNA_1 \parallel P_1^* + DNA_0 + P_1^* \parallel P_2.$

As can be seen from Saiki's assay, P_2 , differing by one nucleotide from P_1^* , will not only more selectively hybridize with free P_1^* than with DNA_0 but it will also bind residual P_1^* , thus effectively enabling all background noise, i.e., a signal from P_1^* not associated with the $DNA_1 \parallel P_1^*$ match, to be eliminated. This is not appellant's invention either.

Appellant has not argued that the prior art applied is

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nonanalogous art.³ Therefore, in order to affirm the examiner's rejection under 35 U.S.C. § 103 over Wallace and Saiki, we must find that the combined prior art teachings reasonably would have led persons having ordinary skill in the art to use Saiki's blocking oligomers in competitive hybridization assays. We have no basis on this record to so find. Rather, we find that the purpose for and the order of Saiki's use of a blocking oligomer in a hybridization assay are limited to the specific two-stage assay Saiki describes. Therefore, we need not speculate whether persons having ordinary skill in the art would have considered the assays described by and the general teaching of Wallace relevant to competitive hybridization assays for RNA. We reverse the examiner's rejection because neither Saiki nor any combination of Wallace and Saiki reasonably suggests the use of a probe complementary to target RNA in combination with a blocking probe complementary to RNA which differs from the target RNA by one nucleotide to assay for target RNA in a mixture suspected to contain RNA which differs from the target RNA by only one nucleotide. The prior art does not expressly recognize and reasonably would not have suggested that an excess of a blocking probe would improve the specificity of competitive hybridization assays for target RNA in mixtures of RNA which differ by one

³ We note that Saiki both cites and distinguishes Conner's method (Saiki, col.2, lines 7-45).

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nucleotide. Rather, based on prior art attempts to improve the effectiveness of DNA assays, persons skilled in the art at the time appellant's invention was made more likely would have sought not to improve the specificity of competitive hybridization assays but to eliminate any competitive hybridization by use of stringent conditions.

5. Conclusion

The examiner's rejection of Claims 6, 7, 9 and 10 under 35 U.S.C. § 103 over the combined teachings of Wallace and Saiki is reversed.

REVERSED

Fred McKelvey
FRED E. MCKELVEY, Chief
Administrative Patent Judge)
)
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William F. Smith
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APPENDIX

6. A hybridization assay for discriminating between mixed first and second RNA molecules, said first RNA molecule including a target nucleotide sequence and said second RNA molecule including a mutant sequence which differs by one nucleotide from said target nucleotide sequence

subjecting said mixed first and second RNA molecules to hybridization conditions in the presence of first and second oligonucleotide probes,

said first oligonucleotide probe including a sequence complementary to said target sequence of said first RNA molecule,

said second oligonucleotide probe being non-complementary to said target sequence but complementary to said mutant sequence,

said second probe being present in substantial excess during said hybridization to suppress hybridization of said first probe to said second RNA molecule...