

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

Paper No. 27

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES

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Ex parte PAUL E. MAYRAND

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Appeal No. 1999-1266  
Application No. 08/859,472<sup>1</sup>

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HEARD: August 07, 2001

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Before WILLIAM F. SMITH, ROBINSON, and ADAMS, Administrative Patent Judges.  
ROBINSON, Administrative Patent Judge.

**DECISION ON APPEAL**

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 16 and 17, which are the only claims pending in the application.

Claim 16 is illustrative of the subject matter on appeal and reads as follows:

16. A method for performing combined PCR amplification and hybridization probing comprising the steps of:

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<sup>1</sup> This application is directed to subject matter related to that which is claimed in Application No. 08/826,538, which is the subject of Appeal No. 1999-1436 currently pending before the Board. We have considered these two appeals together.

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contacting a target nucleic acid sequence with PCR reagents, including at least two PCR primers and a polymerase enzyme substantially lacking any 5' ÷ 3' exonuclease activity, and an oligonucleotide probe comprising:

an oligonucleotide;

a fluorescer molecule attached to a first end of the oligonucleotide;

a quencher molecule attached to a second end of the oligonucleotide such that quencher molecule substantially quenches the fluorescer of the fluorescer molecule whatever the oligonucleotide probe is in a single-stranded state and such that the fluorescer is substantially unquenched whenever the oligonucleotide probe is in a double-stranded state; and

a 3' end which is rendered impervious to the 5' ÷ 3' extension activity of a polymerase; and

subjecting the target nucleic sequence, the oligonucleotide probe, and the PCR reagents to thermal cycling sufficient to amplify the target nucleic acid sequence specified by the PCR reagents.

The references relied upon by the examiner are:

Abramson et al. (Abramson)	5,466,591	Nov. 14, 1995
Link et al. (Link) (WO)	9310267	May. 27, 1993
Heller et al. (Heller) (European Patent)	0 229 943	Jul. 29, 1987

Parkhurst et al. (Parkhurst) "Kinetic Studies by Fluorescence Energy Transfer Employing a Double-Labeled Oligonucleotide: Hybridization to the Oligonucleotide Complement and to Single-Stranded DNA," Biochemistry, Vol. 34, pp. 285-292 (1995)

### **Ground of Rejection**

Claims 16 and 17 stand rejected under 35 U.S.C. § 103. As evidence of obviousness, the examiner relies upon Link, Parkhurst, Heller and Abramson.

We reverse.

### **Background**

The applicant describes the presently claimed invention at page 3 of the specification as being directed to methods and reagents useful for the combined amplification and hybridization probe detection of amplified nucleic acid target sequences in a single reaction vessel. At page 4, applicant describes a method and reagents for the amplification and probe detection where the polymerase is not required to have 5'->3' exonuclease activity.

### **Discussion**

#### **Claim Interpretation**

Claim 16 is directed to a method for performing combined PCR amplification and hybridization probing comprising contacting the target nucleic acid sequence with PCR reagents, including at least two PCR primers, in combination with an oligonucleotide probe in the presence of a polymerase enzyme substantially lacking any 5'->3' exonuclease activity. The claim provides that the oligonucleotide probe has a fluorescer molecule attached to a first end and a quencher molecule attached to a second end such that the

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quencher molecule substantially quenches the fluorescence of the fluorescer molecule whenever the oligonucleotide probe is in a single stranded state and such that the fluorescer is substantially unquenched when the oligonucleotide is in a double-stranded state. In addition, the claim requires that the 3' end of the probe is rendered impervious to the 5'->3' extension activity of a polymerase. The combination is subjected to thermal cycling sufficient to amplify the target nucleic acid sequence specified by the PCR reagents.

The rejection under 35 U.S.C. § 103

In reaching our decision in this appeal, we have given careful consideration to the appellant's specification and claims and to the respective positions articulated by the appellant and the examiner. We make reference to the Examiner's Answer of July 17, 1998 (Paper No. 17) for the examiner's reasoning in support of the rejection and to the appellant's Appeal Brief filed June 10, 1998 (Paper No. 15) for the appellant's arguments thereagainst.

Obviousness is a legal conclusion based on the underlying facts. Graham v. John Deere Co., 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966); Continental Can Co. USA, Inc. v. Monsanto Co., 948 F.2d 1264, 1270, 20 USPQ2d 1746, 1750 (Fed. Cir. 1991); Panduit Corp. v. Dennison Mfg. Co., 810 F.2d 1561, 1566-68, 1 USPQ2d 1593, 1595-97 (Fed. Cir. 1987). Here, the dispositive question is whether it would have been obvious to one of ordinary skill in this art at the time of the invention to carry out a PCR reaction using at least two PCR

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primers in the presence of a hybridization probe which is an oligonucleotide as defined in claim 16 in the presence of a polymerase enzyme substantially lacking any 5'->3' exonuclease activity.

It is well-established that before a conclusion of obviousness may be made based on a combination of references, there must have been a reason, suggestion, or motivation to lead an inventor to combine those references. Pro-Mold & Tool Co. v. Great Lakes Plastics, Inc., 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1629 (Fed. Cir. 1996) (citation omitted). Moreover, the prior art must also establish that one would have had a reasonable expectation of achieving the present invention, *i.e.*, a reasonable expectation of success. In re Vaeck, 947 F.2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion and the reasonable expectation of success must be found in the prior art, not in appellant's disclosure. In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

The examiner relies on Link as disclosing a (Answer, page 4):

method for combined PCR amplification and hybridization probing comprising the steps of contacting a target sequence with two PCR primers, a polymerase such as Taq which lacks 3' to 5' exonuclease activity (page 13, lines 31-37 and including an oligonucleotide probe which was doubly labeled (page 13, line 1) which probes "are not able to act as primers in the PCR or other primer directed reactions (page 11, lines 12-14)" and subjecting the mixture to thermal cycling to amplify target sequence (page 13, lines 31-37).

The examiner acknowledges that Link does not teach (id.):

the use of the specific fluorescent donor and quencher in double stranded states. Link also does not teach the use of a 5'-3' exonuclease deficient polymerase enzyme.

The examiner cites Parkhurst as teaching "the use of doubly labeled oligonucleotide probes in which the probes are quenched in a single stranded state and unquenched in a double stranded state (291, figure 3)." (Id.). The examiner, additionally, cites Heller as teaching "the use of doubly labeled oligonucleotide probes in which the probes are quenched in a single stranded state and unquenched in a double stranded state (page 19, Table A)." (Id.). Abramson is relied on as teaching "the use of 5'-3' exonuclease deficient polymerases in PCR (columns 2-8 and especially, column 7, lines 52-57)." (Answer, page 5).

The examiner concludes that (Answer, pages 5):

[i]t would have been *prima facie* obvious to one having ordinary skill in the art at the time of the invention was made to combine the PCR amplification and hybridization method of Link with the labels of either Parkhurst or Heller since Parkhurst states "The double-labeled oligomer is very effective in signaling hybridization (page 292, column 1, paragraph 2)." . . . It would further have been obvious to combine the method of Link in view of either Parkhurst or Heller with the use of exonuclease deficient polymerase as taught by Abramson since Abramson states "When utilized in a PCR process with double-stranded primer template complex, the 5' to 3' exonuclease activity of a DNA polymerase may result in degradation of the 5'-end of the oligonucleotide primers. This activity is not only undesirable in PCR, but also in second strand cDNA synthesis and sequencing processes (column 7, lines 52-57)". Abramson solves the problem of exonuclease activity by eliminating the exonuclease activity, as stated "Thus, one aspect of this invention involves the generation of thermostable DNA polymerase mutants displaying greatly reduced, attenuated or completely eliminated 5' to 3' exonuclease activity.

What is missing from the examiner's analysis and review of the facts before us, is any direction or suggestion to be found in the prior art, including Abramson, to select the particular enzyme required by claim 16, from those described by Abramson, for use in a PCR hybridization process as presently claimed. As pointed out by appellant (Brief, page 6):

Abramson teaches "DNA polymerases which have been altered or mutated such that a different level of 5' to 3' exonuclease activity is exhibited from that which is exhibited by the native enzyme." . . . That is, Abramson teaches DNA polymerases which have both attenuated and enhanced 5'->3' exonuclease activity.

Taken as a whole, we agree with appellant that "Abramson's teaching is . . . somewhat ambiguous." (Brief, page 6). However, in the only situation where Abramson addresses which enzyme to use in a hybridization reaction in a homogenous assay as presently claimed, Abramson states (column 32, lines 39 through column 33, line 8):

[t]he thermostable DNA polymerases of the present invention which have increased or enhanced 5' to 3' exonuclease activity are particularly useful in the homogeneous assay system . . . which generates signal while the target sequence is amplified, thus, minimizing the post-amplification handling of the amplified product which is common to other assay systems. Furthermore, a particularly preferred use of the thermostable DNA polymerase with increased 5' to 3' exonuclease activity is in a homogeneous assay system which utilizes PCR technology.

The examiner urges that (Answer, paragraph bridging pages 6-7):

[t]he specific teaching cited by Appellant is, in fact, directed towards a homogenous assay in which Abramson states "nucleic acid polymerase having a 5' to 3' nuclease activity under conditions sufficient to permit the 5' to 3' nuclease activity of the polymerase to cleave the annealed, labeled oligonucleotide and release labeled fragments." This homogenous assay it

dramatically different than that of Link and an ordinary practitioner, faced with the choice of which Abramson teaching would be useful in the method of Link in view of Parkhurst or Heller, in which detection proceeds by FRET and not by digestion of labeled oligonucleotides, would choose the teaching that eliminated the 5' to 3' exonuclease . . . since this would not enhance the detection, unlike the second teaching which would abolish detection.

To the extent that the process of Abramson may differ from the claimed process in the use of labeled oligonucleotides versus FRET, it remains that the explicit teaching of Abramson would direct one of ordinary skill away from the use of a polymerase with attenuated or reduced 5' to 3' exonuclease activity as presently claimed. Further, the examiner points to no facts or evidence, in the prior art, which would reasonably establish that one of ordinary skill in this art, with no knowledge of the presently claimed invention, would appreciate the significance of this difference and then select a different enzyme based on this difference. The examiner has pointed to no facts to be found in Link, Parkhurst or Heller which would reasonably be read to have directed one of ordinary skill in the use of a particular enzyme from those disclosed by Abramson.

In order to establish a prima facie case of obviousness on the facts before us, the examiner must have provided evidence which would have led one of ordinary skill in this art, at the time of the invention, to use a polymerase enzyme substantially lacking any 5'->3' exonuclease activity in a combined PCR amplification and hybridization process as presently claimed. On this record, the examiner has not provided facts or substantive

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evidence which would have reasonably suggested going against the explicit teaching of Abramson in a manner which would result in the presently claimed method.

The initial burden of presenting a prima facie case of obviousness rests on the examiner. In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). The examiner's rejection of the claims is fatally defective since they do not properly account for and establish the obviousness of the subject matter as a whole. On these circumstances, we conclude that the examiner has failed to provide the evidence which would reasonably to support a conclusion that the present claims were prima facie obviousness within the meaning of 35 U.S.C. § 103. Where the examiner fails to establish a prima facie case, the rejection is improper and will be overturned. In re Fine, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir.1988). Therefore, the rejection of claims 16 and 17 under 35 U.S.C. § 103, as unpatentable over the combination of Link, Parkhurst, Heller, and Abramson is reversed.

### **Summary**

To summarize, the examiner's rejection of claims 16 and 17 under 35 U.S.C. § 103 is reversed.

**REVERSED**

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Administrative Patent Judge	)	
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	)	BOARD OF PATENT
Douglas W. Robinson	)	
Administrative Patent Judge	)	APPEALS AND
	)	
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