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D E C I S I O N
of 23 January 2002

Case Number: T 1118/98 - 3.3.4

Application Number: 91908846.8

Publication Number: 0520039

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:

A process for distinguishing nucleic acids on the basis of nucleotide differences

Patentee:

E. I. DU PONT DE NEMOURS AND COMPANY

Opponent:

Syngenta Participations AG

Headword:

Nucleic acids/DU PONT DE NEMOURS

Relevant legal provisions:

EPC Art. 123(2), 87, 54, 56

Keyword:

"Main request - added subject-matter (no)"
"Right to priority (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:

T 0514/88; T 0187/91; T 0288/92; T 0746/94; T 0506/95

Catchword:

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Case Number: T 1118/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 23 January 2002

Appellant: Syngenta Participations AG
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Decision under appeal: **Decision of the Opposition Division of the
European Patent Office posted 8 October 1998
rejecting the opposition filed against European
patent No. 0 520 039 pursuant to Article 102(2)
EPC.**

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
V. Di Cerbo

Summary of Facts and Submissions

I. The appeal lies from the decision of the Opposition Division to maintain as granted the patent No. 0 520 039 (application number 91 908 846.8) with the title " A process for distinguishing nucleic acids on the basis of nucleotide differences." and claiming priority rights from 14 March 1990 (US 494 258).

Granted claim 1 read as follows:

" 1. A process for detecting polymorphisms on the basis of nucleotide differences in random segments of nucleic acids comprising:

(a) separately performing an extension reaction on a random segment of each of at least two nucleic acids from different sources, said reaction comprising:

(i) contacting each of the above nucleic acids with at least one nonspecifically targeted, random oligonucleotide primer of greater than 7 nucleotides and extending the primer in an extension reaction whereby for at least one nucleic acid, a random extension product of at least one primer is synthesized; and

(b) comparing the results of separately performed random extension reactions for differences."

Dependent claims 2 to 11 related to further features of the process of claim 1.

II. Oral proceedings were summoned. With their letter dated 23 November 2001, the Appellants (Opponents) informed the Board that they would not take part in the oral proceedings which were held on 23 January 2002.

III. With their letters filed on 27 April 2000 and 18 January 2002, the Respondents (Patentees) filed auxiliary requests (a) to (d) and (e) to (i), respectively.

IV. The documents mentioned in the present decision are:

(3): Welsh, J. and McClelland, M. ; Nucleic Acids Research, Vol. 18, No. 24, pages 7213 to 7218, 1990,

(7): Dear, P.H. and Cook, P.R. ; Nucleic Acids Research, Vol. 17, No. 17, pages 6795 to 6798, 1989,

(10): Williams, J. et al., Nucleic Acids Research, Vol. 18, No. 22, pages 6531 to 6535, 1990,

(11): Skolnick, M.H. and Wallace, R.B., Genomics, Vol. 2, pages 273 to 279, 1988,

(12): Weber, J.L. and May, P.E., Am.J.Hum.Genet., Vol. 44, pages 388 to 396, 1989.

V. The submissions in writing by the Appellants can be summarized as follows:

Claim 1

Article 123(2) EPC; added subject-matter

The term "non-specifically targeted, random" defining the oligonucleotide primer in process step (i) of claim 1 extended the claimed subject-matter beyond the content of the application as filed:

- primers with **known** nucleotide sequences were used all through the description, which was contrary to the notion of the primer being random ie being an oligonucleotide molecule of any possible length and sequence.
- once an oligonucleotide primer was identified as detecting a polymorphism, it was that very same specific primer which had to be used again and again to reproduce the result.
- the term "random" was not mentioned in the description as filed in connection with the primers but in connection with the nucleic acid segment acting as the target.

Article 87 EPC, Article 54(2) EPC; priority rights, novelty

The amendments relating to the definition of the oligonucleotide primer did not appear in the priority document, meaning that priority could not be validly claimed. Therefore, for the purpose of Article 54(2) EPC, it was the date of filing of the application (12 February 1991) which counted. Each of documents (3) and (10) which were published before that date and disclosed processes with the same technical features as claim 1 was, thus, novelty destroying for the subject-matter of said claim.

Article 56 EPC; inventive step

The restriction fragment length polymorphism (RFLP) technique which was the usual way to screen for DNA polymorphisms was the closest prior art: it involved the digestion of the DNAs to be compared with restriction endonucleases followed by analysis of the resulting fragments.

Starting from this closest prior art, the technical problem to be solved was the provision of an alternative method for detecting polymorphisms.

Alternative methods for that purpose were already known in the prior art from document (11) or document (12). These latter methods differed from the claimed one only in that the structure of the polymorphisms was at least partially defined by sequence and, therefore, the primers could be predesigned according to these sequences. That in the absence of detailed sequence information, some oligonucleotides of "random" sequence would be able to amplify sequences which can serve as copies of markers had already been disclosed in detail in document (7). Though document (7) realized that certain problems would be associated with the random primed technique when used for linkage mapping, it did not teach that the technique did not work for the genotyping of closely related organisms, which generally relied on the detection of polymorphisms.

Thus, the combination of the RFLP technique with the teachings of document (11) or (12) and of document (7) rendered the claimed subject-matter obvious.

Alternatively, documents (11) or (12) could be regarded

as the closest prior art. The technical problem to be solved would then be the detection of genetic polymorphisms in **random** nonspecific nucleic acid segments. The solution given to this problem was rendered obvious by the combination of the closest prior art and of document (7).

VI. The submissions in writing and during oral proceedings by the Respondents can be summarized as follows:

Claim 1

Article 123(2) EPC; added subject-matter

To determine whether the expression "nonspecifically targeted, random oligonucleotide" had a basis in the application as filed, it was necessary to take into account the teachings of the original disclosure **as a whole**. In particular, the way the primer was said to operate was of paramount importance: the primer was described as binding to a random segment of the nucleic acid template and this implied that the priming process was random ie that the primer itself was random and nonspecifically targeted.

The fact that in the examples provided, the primers were identified by their sequences did not mean that they were not random. They were random because they had not been designed with reference to the target sequence, they bound at random sites and could be of any length and nucleotide composition compatible with efficiency of priming.

It was true that in the application as filed the term "arbitrary" was used in relation to the primer rather

than the term "random". Yet, on page 6, lines 26 to 28, "arbitrary " was defined as meaning "generated by computer or selected at random from a gene bank", thus, the terms "arbitrary" and "random" carried the same meaning.

The requirements of Article 123(2) EPC were fulfilled.

Article 87 EPC, Article 54(2) EPC; priority rights, novelty

The priority document and the application as filed were identical in wording. As the application as filed disclosed a nonspecifically targeted, random primer, so did the priority document. Priority was, thus, valid. For the purpose of Article 54(2) EPC, the filing date of the patent in suit was its priority date.

Documents (3) and (10) were post-published and, thus, their respective teachings could not destroy novelty.

Article 56 EPC; inventive step

Documents (11) or (12) allowed the identification of polymorphisms **if** the sequences surrounding the polymorphic site were known to the extent of allowing the design of specific primers. In contrast, the essence of the invention was to allow identification and analysis of polymorphisms **without** the prior knowledge of the surrounding nucleotide sequences.

There was no motivation for combining the teachings of either document (11) or (12) with that of document (7) which was a theoretical method of linkage mapping having as sole similarity to the present method that random primers were used. Document (7) was absolutely

silent as to the possible utilisation of polymorphic random primer amplification products for genetic mapping. The authors even predicted that polymorphisms would be detrimental for their own purpose. Combining the teachings of document (11) or (12) with those of document (7) could only result from **ex post facto** analysis which was not allowable in the assessment of inventive step.

VII. The Appellants had requested in writing that the decision under appeal be set aside and that the patent in suit be revoked.

The Respondents requested that the appeal be dismissed and that the patent be maintained as granted (main request), or that the decision under appeal be set aside and a patent be maintained on the basis of one of the auxiliary requests (a) to (d) filed on 27 April 2000 and (e) to (i) filed on 18 January 2002.

Reasons for the Decision

Claim 1

Article 123(2) EPC; added subject-matter

1. The objection raised by the Appellants under Article 123(2) EPC is that whereas claim 1 was amended to refer to a "**nonspecifically targeted, random**" oligonucleotide primer, the application as filed did not disclose such a primer. Indeed, the expression "nonspecifically targeted, random primer" is not to be found *expressis verbis* in said application, where the

primer is rather defined as being "**arbitrary**".

2. In accordance with the case law of the Boards of Appeal such as, for example, decisions T 514/88 (OJ EPO 1992, 570), T 288/92 of 18 November 1993) and T 187/91 (OJ EPO 1994, 572), an amendment is allowable under Article 123(2) EPC if it can be directly and unambiguously derived from the application as filed. In the present case, it is, thus, necessary to decide whether the term "arbitrary" which carries within it the meaning of "depending on choice" as well as "selected at random" is to be unambiguously understood on the basis of the originally filed disclosure as "nonspecifically targeted, random".

3. An arbitrary primer is defined on page 6, lines 26 to 28 of the application as filed as "conveniently... generated by computer or selected at random from a gene bank". Such methods do not impose any restrictions on the specific alignment of the nucleotides within the primer. In example I, eight primers are defined by their sequences which contain the same proportion of the bases A and T as the bases G and C "for generally equal stability when attached to the template" ie which exhibit a property expected of efficient primers in general. This property, like the methods which were just mentioned, does not impose any restrictions on the specific alignment of the nucleotides within the primer. The application as filed thus conveys the information that an arbitrary primer is **not** to be defined by its sequence, otherwise stated, that it is of any sequence ie **that it is random**.

4. Furthermore, document (10) (taken as an expert document) provides indirect evidence that the person

skilled in the art considered the words "arbitrary" and "random" to have the same meaning since in this document both words are used interchangeably to characterise primers (Abstract and page 6533, right hand column, 2nd paragraph).

5. All through the application as filed, the sequence of the **target** is qualified as being "random", no specific target sequences are disclosed in the examples. This implies that the sequence of the primers recognizing the target cannot be pre-determined as a function of the sequence of said target ie that the primer is **nonspecifically targeted**.

6. The Appellants argued that because the primers used in the examples in the application as filed were defined by their sequences, they could not be regarded as random. The Board, however, does not find this argument convincing. The only definite way to identify a DNA fragment (whether or not it be a primer) is by its sequence, as was indeed done in the original disclosure. Yet, for a sequence **not to be random**, it is not sufficient that the alignment of nucleotides within it be known, to the contrary, it is necessary that a wilful choice is made on the basis of criteria, whatever they are, as to what should be the **succession** of nucleotides in the alignment. The Appellants failed to provide any evidence that this step was taken in deciding the sequences of the exemplified primers, nor can the Board find in the disclosure as filed as a whole that such a step was intended. The mentioning on page 6 that the primer sequences is generated by computer or selected at random from a gene bank clearly supports the idea that it was not.

7. It was also argued that once a primer had been shown to identify a polymorphism, it was to be used again and again when wanting to re-identify the same polymorphism and that, in that sense, the primer was not random. This approach is not relevant because once a polymorphism is identified, it does not qualify any more as being a random polymorphism and, thus, the fact of always using the same primer fo re-identify it is no evidence that primers used in connection with finding random polymorphisms are not random.
8. The gist of Article 123(2) EPC is that the public must not be taken by surprise by claims which it could not directly and unambiguously have expected on the basis of the original disclosure in the application as filed (see decision T 746/94 of 5 November 1998). In the Board's judgment, and for the reasons given in points 3 to 5 above, the skilled person would understand that within the context of the application as filed, the expression "arbitrary primer" has the same meaning as the expression "nonspecifically targeted, random primer" and, thus, would not be taken by surprise by the claimed subject-matter.
9. The requirements of Article 123(2) EPC are fulfilled.

Article 87 EPC, Article 54 EPC; priority rights, novelty

10. It is not disputed that the priority application and the application as filed have the same wording. Taking into account the finding in point 8 above that the application as filed discloses nonspecifically targeted, random primers, the same must be true of the priority application. Priority is, thus, valid. Documents (3) or (10) which were published after the

priority date may not be taken into account for the purpose of assessing novelty. There are no documents on file pre-dating the priority date disclosing a process such as claimed.

11. Novelty is acknowledged.

Article 56 EPC; inventive step

12. The first step in the assessment of inventive step is to identify the closest prior art. According to the Appellants, the RFLP method or the teachings of documents (11) or (12) could equally serve in this respect. At the priority date, the RFLP method was already part of the common general knowledge of the person skilled in the art (see patent in suit, page 3, lines 21 to 26). It enables the characterisation of mutations (DNA polymorphisms) in DNA fragments, the sequence of which is not known (**random DNA fragments**). Documents (11) or (12), on the contrary, describe the genotyping of DNA fragments, the sequences of which are sufficiently **known** that they can be **specifically** amplified.

13. In accordance with the case law of the Boards of Appeal (T 506/95 of 5 February 1997), the closest prior art is that most suitable for the purpose claimed by the invention, not that superficially showing structural similarities with the solution claimed.

14. In the present case, the purpose of the invention is to detect DNA polymorphisms in **random DNA fragments**. Thus, although the claimed method like the methods of documents (11) or (12) makes use of DNA amplification, it is nonetheless the RFLP method which constitutes the

- closest prior art.
15. As already mentioned in point 12 above, the RFLP method consists in digesting random DNA fragments with restriction endonucleases and analysing the resulting fragments. Mutations that affect the recognition sequence of the endonuclease will preclude enzymatic cleavage at that site, thereby altering the cleavage pattern of the DNA. Polymorphisms are detected as differences in the lengths of the restriction fragments.
 16. Starting from the closest prior art, the problem to be solved can be defined as providing another method for the detection of DNA polymorphisms.
 17. The solution given in claim 1 is to use nonspecifically targeted, random primers to amplify random segments of genomic DNA. Polymorphisms are then detected by comparing the results of the amplification reactions obtained with the same random primers hybridizing to the DNA of different organisms.
 18. This approach (random DNA amplification) is based on a totally different rationale from that used in the RFLP method (restriction mapping). In fact, the only document on file which describes a method involving random primers is document (7). This document discloses a **theoretical** approach for linkage mapping the genome. The first step in this approach is to identify the markers to be linked on bulk human DNA: this would be achieved by hybridising two oligonucleotides of random sequence at sites scattered randomly through the genome; some sites could be expected to be about 1 Kd apart so the intervening sequence could be amplified by

- PCR. The amplified intervening sequences characterized by their lengths and the random primers used to obtain them would serve as markers (document (7), page 6797).
19. The Board agrees with the Respondents (point 9.5 of their submissions filed on 27 April 2000 with reference to their submissions of 1 August 1996) that this method inherently relies on the fact that each DNA sample analysed for mapping consists of **identical DNA sequences** as any polymorphisms between these sequences would distort the data used to determine the degree of linkage. This is also directly derivable from document (7) itself where it is stated on page 6801, line 12 that "the presence of polymorphisms will cause significant loss". In the Board's judgment, the skilled person would not find in the teachings of document (7) any incentive to use random priming in a method such as claimed which, contrary to that of document (7), is devoted to detecting **differences in DNA sequences** (DNA polymorphisms).
20. It was argued that the combination of the RFLP method with the teachings of documents (11) or (12) and of document (7) rendered obvious the claimed subject-matter. Documents (11) and (12) describe processes for detecting particular polymorphisms, the sequences of which **are known** (see document (11) page 276, "candidate loci" and document (12), Summary). In the course of these processes, the known polymorphisms are amplified by PCR with the help of primers, the sequence of which is **pre-determined** on the basis of that of the target DNA. The sole similarity with the presently claimed process is the use of the PCR reaction. The Board fails to see how this similarity would suggest to the person

skilled in the art to use **random primers**.

21. In summary, the RFLP method is not about detecting random polymorphisms by DNA amplification. In document (7), DNA polymorphisms are identified as a hindrance to the successful outcome of the then disclosed theoretical approach involving random priming. The teachings of documents (11) or (12) do not serve to arrive at the claimed invention. It is, thus, concluded that the invention is not rendered obvious by the prior art.

22. Inventive step is acknowledged.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairwoman:

P. Cremona

U. Kinkeldey