BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [X] To Chairmen
(D) [ ] No distribution

Datasheet for the decision of 30 July 2010

Case Number: T 0642/07 - 3.3.04

Application Number: 00959909.3

Publication Number: 1220949

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Chromosome-wide analysis of protein-DNA interactions

Applicant:
WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH

Headword:
Chromosome-wide analysis/WHITEHEAD INSTITUTE

Relevant legal provisions:
EPC Art. 123(2), 56

Relevant legal provisions (EPC 1973):
-

Keyword:
"Added subject-matter (no)"
"Inventive step (yes)"

Decisions cited:
-

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0642/07 - 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 30 July 2010

Appellant: WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH
Nine Cambridge Center
Cambridge, MA 02142 (US)

Representative: Ellis, Katherine Elizabeth Sleigh
Williams Powell
Staple Court
11 Staple Inn Buildings
London, WC1V 7QH (GB)

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 20 November 2006 refusing European application No. 00959909 pursuant to Article 97(1) EPC 1973.**

Composition of the Board:

Chairman: M. Wieser
Members: R. Gramaglia
D. S. Rogers

Summary of Facts and Submissions

I. The appeal lies from the decision of the Examining Division issued on 20 November 2006, whereby European patent application No. 00 959 909.3, published as WO-A-01/16378, was refused pursuant to Article 97(1) EPC (1973). The reason given by the examining division for refusal was that the claims of the main request then on file infringed Article 123(2) EPC, whereas those of the first and second auxiliary requests then on file lacked inventive step.

II. Claim 1 of the second auxiliary request refused by the examining division read as follows:

"1. An in vitro genome-wide location profiling method of identifying a region of a genome of a living cell to which a protein of interest binds, comprising the steps of:

a) cross-linking DNA binding protein in the living cells to genomic DNA of the living cell, thereby producing DNA binding protein cross-linked to genomic DNA;

b) generating DNA fragments of the genomic DNA cross-linked to DNA binding protein in a), thereby producing a mixture comprising DNA fragments to which DNA binding protein is bound;

c) removing the fragment (one or more) to which the protein of interest is bound from the mixture produced in b);

d) separating the DNA fragment (one or more) obtained in c) from the protein of interest;

e) blunting the ends of the DNA fragment obtained in d);

f) ligating the blunt ended fragment (one or more) obtained in e) to a unidirectional linker;

g) amplifying the DNA fragment (one or more) of f) using a ligation mediated polymerase chain reaction strategy;

h) fluorescently labelling the DNA fragment (one or more) of (g) with a first fluorophore during step (g);

i) generating a control by amplifying a sample of DNA that has not been enriched in accordance with step c) using LM-PCR in the presence of a second fluorophore;

j) combining the labelled DNA fragment (one or more) of (h) with a DNA micro-array comprising a sequence complementary to genomic DNA of the cell under conditions in which hybridisation between the DNA fragment (one or more) and a region complementary to the genomic DNA occurs;

k) combining the labelled DNA fragment of (i) with the same DNA micro-array used in step (j) under conditions in which hybridisation between the DNA fragments and a region complementary to the genomic DNA occurs;

l) identifying the region of the sequence complementary to genomic DNA to which the DNA fragment (one or more)

hybridise by measuring the fluorescence intensity of the first chromophore;

m) comparing the fluorescence intensity measured in step (l) with the fluorescence intensity of the control, whereby the fluorescence intensity in a region of the genome which is greater than the fluorescence intensity of the control in the region indicates the region of the genome in the cell in which the protein of interest binds."

III. The following documents are referred to in the present decision:

D1 Blat Y. et al., Cell, Vol. 98, pages 249-259 (1999);

D2 Hecht A. et al., Nature, Vol. 383, pages 92-96 (1996);

D3 Barany F., PCR Methods and Applications, Vol. 1, pages 5-16 (1991);

D40 Strutt H. et al., The EMBO J., Vol. 16 (12), pages 3621-3632 (1997);

D41 Roberts C.J. et al., Science, Vol. 287, pages 873-880 (4 February 2000).

IV. In its decision to refuse the second auxiliary request, the examining division considered document D1 to represent the closest prior art for the method according to claim 1 then on file and considered that the latter differed from that disclosed in document D1

by features (e) to (g) (relating to ligation-mediated PCR) and features (h) to (m) (reflecting dual fluorescent labelling and analysis of the signals on a single array). It was further the opinion of the examining division that the Examples in the application did not relate or disclose a combination of ChIP (Chromatin Immunoprecipitation), LM-PCR (Ligation-mediated PCR) and fluorescent labelling with two different fluorophores on a single DNA array, let alone any technical effect associated with using dual fluorescent labelling and analysis of the signals on a single array.

Starting from document D1 as closest prior art, the examining division thus viewed the technical problem to be formulated as the provision of an improved method for an in-vitro genome wide location profiling requiring, in comparison to conventional PCR, less knowledge of the target nucleic acid to be amplified and which, moreover, avoided radioactivity. The examining division decided that features (e) to (g) in claim 1 (relating to ligation-mediated PCR) did not involve an inventive step in the light of document D3, disclosing ligation-mediated PCR. Features (h) to (m) in claim 1 (reflecting dual fluorescent labelling and analysis of the signals on a single array) were also found to be obvious, since the advantages of replacing radioactive labels with fluorescent labels were known to the skilled person.

V. The applicant (appellant) filed an appeal against the decision of the examining division. The Statement of Grounds of Appeal included inter alia evidence (Annexes I to XIX) in support of the appeal.

VI. In a communication dated 30 July 2008, the board expressed its preliminary opinion and invited the appellant to take position as regards documents D40 and D41 which came to the board's attention upon reviewing the appellant's Annexes I to XIX.

VII. On 10 August 2009 the board issued a further communication. In response thereto the appellant submitted with the letter dated 9 September 2009 an amended set of claims 1 to 6 as the sole request, of which claim 1 read as follows:

"1. An in vitro genome-wide location profiling method of identifying a region of a genome of a living cell to which a protein of interest binds, comprising the steps of:

a) cross-linking DNA binding protein in the living cells to genomic DNA of the living cell, thereby producing DNA binding protein cross-linked to genomic DNA;

b) generating DNA fragments of the genomic DNA cross-linked to DNA binding protein in a), thereby producing a mixture comprising DNA fragments to which DNA binding protein is bound;

c) removing the fragment (one or more) to which the protein of interest is bound from the mixture produced in b);

d) separating the DNA fragment (one or more) obtained in c) from the protein of interest;

e) amplifying the DNA fragment (one or more) of d) using a ligation mediated polymerase chain reaction strategy; characterised in that the method further comprises the steps of:

f) fluorescently labelling the DNA fragment (one or more) of (e) with a first fluorophore during or after step (e);

g) generating a control by amplifying a sample of DNA that has not been enriched in accordance with step c) using LM-PCR in the presence of a second fluorophore;

h) combining the labelled DNA fragment (one or more) of (f) with a DNA micro-array comprising a sequence complementary to genomic DNA of the cell under conditions in which hybridisation between the DNA fragment (one or more) and a region complementary to the genomic DNA occurs;

i) combining the labelled DNA fragment of (g) with the same DNA micro-array used in step (h) under conditions in which hybridisation between the DNA fragments and a region complementary to the genomic DNA occurs;

j) identifying the region of the sequence complementary to genomic DNA to which the DNA fragment (one or more) hybridise by measuring the fluorescence intensity of the first chromophore;

k) comparing the fluorescence intensity measured in step (j) with the fluorescence intensity of the control, whereby the fluorescence intensity in a region of the

genome which is greater than the fluorescence intensity of the control in the region indicates the region of the genome in the cell in which the protein of interest binds."

Dependent claims 2 to 6 related to specific embodiments of the method according to claim 1.

VIII. The submissions by the appellant (applicant), insofar as they are relevant to the present decision, can be summarized as follows:

Article 123(2) EPC

– The wording of amended claim 1 was almost identical to that of claim 1 of the second auxiliary request considered by the examining division at oral proceedings. Basis for this claim could be found at page 2, lines 2 to 3 and page 5, line 15 of the published WO application, where the genome wide applicability of the method was discussed, and also at page 9, lines 25 to 30, where the use of two different fluorophores to identify the region (or regions) of genomic DNA to which the protein of interest binds was disclosed.

Inventive step

– The present inventors have discovered that by combining ChIP (Chromatin Immunoprecipitation), LM-PCR (Ligation-mediated PCR) and dual fluorescent labelling on a single DNA array, it was possible not only to determine the nature of the protein-DNA interactions across the whole genome, but also to

obtain highly reproducible results, wherein the signals for more than 99.9% of genes were identical within the error range using independent samples of 1 ng of genomic DNA.

– Moreover, the combination of features characterising the method of the present invention has resulted in a technique that has become the method of choice of the majority of researchers investigating the protein-DNA interactions within genomes.

– The method of the present invention has been found to satisfy a long felt need within the industry for the ability to perform a successful genome wide analysis of protein-DNA interactions using small sample sizes for both simple and complex genomes. The successful application of this technique could best be appreciated in the light of the large number of publications, which reference its use. A selection of documents detailing the use of the technique of the present invention was listed in Annexes II to XVIII and in the protocol used commercially by NimbleGen Systems (Annex XIX).

– The problem addressed by the present invention together with its solution has been neither taught nor suggested by any prior art document, including documents D1 or D3, or by the combination thereof and would thus not have been obvious to a skilled person.

– The use of PCR using non-specific primers (Ligation-mediated PCR) allowed the skilled person to analyse chromosome protein-DNA interactions for unknown DNA

fragments using relatively small samples. This was not possible using conventional PCR techniques, which employed gene-specific primers. It would not have been obvious to a skilled person at the priority date of the invention that genome-wide amplification of sequences using non-gene specific primers would facilitate the provision of a sensitive and reproducible method of evaluating the binding of proteins of interest across a sample of nucleic acids representing an entire genome.

IX. The appellant (applicant) requested that the decision under appeal be set aside and the patent be granted on the basis of claims 1 to 6 filed with its letter dated 9 September 2009.

Reasons for the Decision

Article 123(2) EPC

1. The examining division did not raise any objection under Article 123(2) EPC against the claims of the second auxiliary request then before it (see paragraph II supra), and the board also sees no objections. The only differences between the present claims submitted with the letter dated 9 September 2009 and those of the second auxiliary request considered by the examining division lie in (1) the omission (and the overall step renumbering) in new claim 1 of steps e) and f) of former claim 1, and (2) the addition of the term "or after" in step f) of new claim 1. Claim 2 to 6 of the amended claims are identical to claim 2 to 6 of the

second auxiliary request considered by the examining division.

Feature (1) above is based on claim 9 of the application as published, where no such steps e) ("blunting the ends of the DNA fragment obtained in d)") and f) ("ligating the blunt ended fragment (one or more) obtained in e) to a unidirectional linker") were present.

As regards feature (2) above, the wording "or after" finds a basis on page 2, lines 28-29 ("and then fluorescently labelled") of the application as published, according to which fluorescent labelling occurs after amplification.

In conclusion, the claims submitted with the letter dated 9 September 2009 do not infringe Article 123(2) EPC.

Novelty (Article 56 EPC)

2. No document of the prior art discloses a method according to claim 1 for identifying a region of a genome to which a protein of interest binds, which method relies on the combination of the three techniques ChIP, LM-PCR and fluorescent labelling with two different fluorophores on a single DNA array. This was also the view of the examining division.

Inventive step (Article 56 EPC)

3. The method according to claim 1 for identifying a region of a genome to which a protein of interest binds

relies on the combination of three techniques, namely (i) ChIP (Chromatin Immunoprecipitation: see page 9, lines 22-23 of the present application and steps (a) to (d) in claim 1; (ii) LM-PCR (Ligation-mediated PCR: see page 9, lines 4-6 and steps (e) and (g) in claim 1) and (iii) fluorescent labelling with two different fluorophores on a single DNA array (see page 9, lines 8-12 and 29-31 and steps (f) to (k) in claim 1). Each of these techniques can be summarized as follows:

Chromatin Immunoprecipitation (ChIP)

4. The ChIP is a technique wherein proteins which bind to DNA in a cell are crosslinked (e.g. with formaldehyde). The cells are collected by centrifugation, lysed and the DNA is sheared (e.g., by sonication). The resulting mixture includes DNA fragments of the genome to which the protein of interest is bound (by crosslinking) and fragments devoid of the protein. The protein-bound DNA fragments are removed from the mixture by immunoprecipitation. In this immunoprecipitated fraction, the protein is then released from the DNA fragments by "crosslink reversal" (see page 2, line 27 of the application).

Amplification of the immunoprecipitated DNA via a ligation mediated polymerase chain reaction (LM-PCR)

5. Since the yields from the immunoprecipitation technique are relatively small, the immunoprecipitated DNA undergoes amplification (and labelling: see point 5 below) before its use as a probe in hybridization experiments for identifying the searched for binding site(s). The amplification technique according to steps

(e) and (g) of present claim 1 (see also page 2, line 28) is a ligation mediated polymerase chain reaction (LM-PCR). This strategy combines the use of a specific primer with a nonspecific primer (hybridizing to a newly ligated linker). In other words, this technique enables the amplification of a DNA sequence wherein only one end is known.

Dual fluorescent labelling on a single micro-array

6. During LM-PCR amplification, the DNA fragments from the immunoprecipited pool are fluorescently labelled with a first colour, whereas control (non-immunoprecipitated) DNA fragments are fluorescently labelled with a second colour. The signals are then analysed on a single DNA micro-array, whereby the ratio immunoprecipitated/ control (non-immunoprecipitated) of fluorescence intensity is directly proportional to the relative binding of the protein of interest to each DNA sequence represented on the array (see page 9, lines 8-12 and 29-31 and page 10, lines 1-4).

Closest prior art

7. The examining division considered document D1 as the closest prior art (see paragraph IV supra). This document discloses a method to determine the binding sites of cohesins along yeast chromosome III (see the title), whereby ChIP is combined with a **conventional** polymerase chain reaction (PCR) amplification technique. The authors of document D1 indeed state on page 250, r-h column, that in standard protocols, the immunoprecipitated DNA is used as a template for PCR. In this context (see document D1, page 250, line 5 from

the bottom), reference is made to document D2. The latter document discloses on page 93, r-h column, last full paragraph, a conventional PCR amplification technique. Conventional PCR relies on the use of specific primer pairs so that known DNA fragments can be amplified. Each PCR primer pair is only capable of amplifying a specific DNA sequence, both sides of which are known.

Once amplified, the DNA is radiolabeled and used to probe membranes containing the chromosome III DNA fragments (see page 258, l-h column, under the chapter headed "Hybridization and Quantitation")

8. However, as alluded to in the board's communication dated 30 July 2008, document D40 discloses (see pages 3622, under the heading "Results" and paragraph bridging pages 3630 and 3631) ChIP in combination with **ligation-mediated** polymerase chain reaction (LM-PCR; see pages 3622 under the heading "Results" and paragraph bridging pages 3630 and 3631). LM-PCR indeed comprises the steps of rendering a nucleic acid fragment blunt ended, ligating to this blunt end a linker and using this linker, together with a target specific primer, as a primer binding site for PCR amplification (see document D3, page 4, column 2, line 23 to column 3, line 6).

According to document D40, the so-amplified DNA is then radiolabeled and used as a probe in a Southern hybridization (see page 3622, r-h column, lines 1-5 under the chapter headed "PC protein binds strongly to discrete sequences of the BX-C").

The claimed method thus **only** differs from that described in document D40 by the dual fluorescent labelling and analysis of the signals on a single array (see features (f) to (k) in claim 1) instead of Southern hybridization with the radiolabeled probes, as in document D40. Therefore, the board considers document D40 as representing the closest prior art underlying the method for identifying the region(s) of a genome to which a protein of interest binds according to claim 1.

9. The examining division (see paragraph 4.4 of the decision under appeal) decided that the Examples in the application as filed failed to disclose a technique according to present claim 1 (i.e., a combination of ChIP, LM-PCR and fluorescent labelling with two different fluorophores on a single DNA array), let alone any technical effect associated with using dual fluorescent labelling and analysis of the signals on a single array. Hence, starting from document D1 as closest prior art, the examining division came to the conclusion that the problem to be solved lay in avoiding the use of radioactive labels. The use of fluorescent labels was the proposed solution to this problem.

However, the board observes that the passage in the published WO application from page 9, line 14 to page 10, line 12 (see also Fig. 1), clearly relates to a method according to claim 1, involving a combination of ChIP, LM-PCR and fluorescent labelling with two different fluorophores on a single DNA array. An image generated by this technique is also shown in Figure 5A. The latter illustrates the claimed method, wherein the

unenriched and the immunoprecipited DNA generate green and red fluorescence, respectively. The spots for which the red intensity is over-represented indicate binding of the targeted protein to these DNA sequences on the array (see page 10, lines 7-8 and page 4, lines 20-23).

As regards the technical effect, it is stated on page 10, lines 9-12 of the published WO application that the claimed method gives highly reproducible results, wherein the signals for more than 99.9% of the genes were identical within the error range using independent samples of the order of 1 ng of genomic DNA. Fig. 5 B illustrates the high reproducibility of the results (Cy3 and Cy5 are the two fluorophores; see also page 10, line 12 and page 4, lines 24-29).

10. Starting from document D40 as closest prior art, in the board's judgement, the technical problem to be formulated is the provision of an improved method for identifying a region of a genome to which a protein of interest binds, such method giving highly reproducible results. This problem is solved by replacing the radiolabeled probe-based Southern hybridization in document D40 (see point 8 supra) with dual fluorescent labelling and analysis of the signals on a single array. In view of the experimental results referred to on page 10, lines 9-12 and in Fig. 5 B of the published WO application, compared with the lower reproducibility reported in document D40 (see the legend to Fig. 2, line 6 from the bottom) and in document D1 (see page 251, r-h column, line 8 from the bottom), the board is satisfied that the above problem has been solved.

11. The relevant question in respect of inventive step is whether or not it was necessary for the skilled person to apply inventive skill in order to arrive at the claimed solution.

12. Document D40 itself did not provide any hint.

The only document before the board describing dual fluorescent labeling and analysis of the signals on a single array is document D41. Document D41 was published on 4 February 2000, i.e., between the priority date (1 September 1999) and the filing date (1 September 2000) of the present application. However, the now claimed subject-matter including dual fluorescent labelling (i.e., with two different fluorophores) on a single DNA array is not disclosed in the priority document of the patent application in suit. Hence, the date for determining the state of the art for the now claimed subject—mater is the fiking date of the application. Thus, document D41 becomes prior art under Article 54(2) EPC.

13. Document D41 discloses on page 2/19 (see lines 1-6 under Fig. 1) dual fluorescent labelling on a single DNA array. The experiment described in document D41 aims at characterizing the genome-wide changes in transcription that accompany pherormone signalling. For this purpose, DNA microarrays including more than 97% of the genes of S. cerevisiae were probed with differentially labelled (Cy3, green; Cy5, red) cDNA pools derived from pherormone-treated or mock-treated cells. However, the board observes that this technique of dual fluorescent labelling on a single DNA array is performed in the context of measuring how much mRNA

transcription is induced by pherormones, which is remote from identifying a region of a genome to which a protein of interest binds. Moreover, document D41 fails to suggest that dual fluorescent labelling on a single DNA array brings about an increased reproducibility of the results.

14. For these reasons, the board concludes that the solution to the problem underlying the application in suit according to claim 1 cannot be derived in an obvious way from a combination of the teachings of document D40 and D41.

The subject-matter of claim 1, and of claims 2-6 depending thereon, involves an inventive step according to the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to grant a patent on the basis of claims 1 to 6 filed on 9 September 2009, and a description to be adapted thereto.

The Registrar: The Chairman:

P. Cremona M. Wieser