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Datasheet for the decision of 11 March 2008

Case Number: T 0547/06 - 3.3.08

Application Number: 97947374.1

Publication Number: 0937097

IPC: C07H 21/00

Language of the proceedings: EN

Title of invention:
COMPOSITIONS AND METHODS FOR IMMOBILIZING NUCLEIC ACIDS TO SOLID SUPPORTS

Patentee:
SEQUENOM, INC:

Opponent:
BioMérieux

Headword:
Nucleic acid coated beads/SEQUENOM

Relevant legal provisions:
EPC Art. 54, 56

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request - novelty (no)"
"First auxiliary request - inventive step (no)"

Decisions cited:
-

Catchword:
-

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Case Number: T 0547/06 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 11 March 2008

Appellant: (Opponent)
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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 14 February 2006 concerning maintenance of European patent No. 0937097 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Heath

Summary of Facts and Submissions

I. European patent No. 0 937 097, based on the International application No. PCT/US97/20194 and published under the PCT as WO 98/20019 with the title "Compositions and methods for immobilizing nucleic acids to solid supports", was granted with 20 claims.

II. The patent was opposed on the grounds as set forth in Articles 100(a),(b) and (c) EPC. The opposition division considered that the main request and the first auxiliary request did not satisfy the requirements of Articles 54 and 56 EPC, respectively. The patent was maintained in amended form based on the second auxiliary request filed on 7 December 2005.

III. The opponent (appellant) filed a notice of appeal, paid the appeal fee and submitted the statement setting out the grounds of appeal in a letter dated 8 June 2006. Further submissions were made in a letter dated 21 June 2006.

IV. With its letter dated 7 November 2006, the patentee (respondent) replied to appellant's grounds of appeal and submissions.

V. The board sent a communication pursuant to Article 11(1) (now Article 15(1) - see OJ EPO 2007, page 543) of the Rules of Procedure of the Boards of Appeal (RPBA) and informed the parties of its preliminary, non-binding opinion on substantive matters.

VI. With letters dated 5 and 11 February 2008, the appellant and the respondent replied, respectively, to

the board's communication. The latter also filed a first and a second auxiliary request.

VII. Oral proceedings took place on 11 March 2008. At the beginning of the oral proceedings, the respondent withdrew its second auxiliary request.

VIII. Claim 1 of respondent's **main request** - which was identical to the second auxiliary request on which the patent had been maintained by the opposition division - read as follows:

"1. A composition, comprising a bead conjugated to a solid support, and further conjugated to a nucleic acid, wherein the solid support is in a from (*sic*) selected from among beads, combs, pins, wafers with pits, arrays of pits, arrays of nanolitre wells, beads in an array of pits and beads in an array of nanolitre wells, and wherein the bead is conjugated to the solid support via ionic or covalent attachment or via hydrophobic, magnetic or polar interaction."

Claims 2 to 7 related to particular embodiments of claim 1. Claim 8 was directed to a process of making a bead conjugated to a solid support and further conjugated to a nucleic acid, comprising a step (a) of conjugating a bead to a nucleic acid and step (b) conjugating a bead to a solid support in a form defined as in claim 1 and, wherein steps (a) and (b) were performed sequentially, in any order or simultaneously and wherein the bead was conjugated to the solid support defined as in claim 1. Claims 9 to 13 related to particular embodiments of claim 8. Claim 14 was directed to a kit comprising i) beads functionalized

for linking nucleic acid to the beads, ii) an insoluble support in a form defined as in claim 1 and iii) conjugation means for linking nucleic acids to the beads and the beads to the support by means of ionic or covalent attachment or via hydrophobic, magnetic or polar interaction.

IX. The **first auxiliary request** differed from the main request by the deletion in independent claims 1, 8 and 14 of the forms "*beads, combs, pins and wafers with pits*". In addition, claim 8 did not define the type of conjugation to the solid support.

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

D1: EP-A-0 420 053 (publication date: 3 April 1991);

D15: Y. Dolitzky et al., Analytical Biochemistry, Vol. 220, 1994, pages 257 to 267;

D25: M.J. O'Donnell-Maloney et al., Trends in Biotechnology, 1996, Vol. 14(10), October 1996, pages 401 to 407.

XI. The arguments of the appellant, insofar as relevant to the present decision, may be summarized as follows:

Main request
Article 54 EPC

Document D1 disclosed solid support systems comprising a support material on which particles coated with a bioaffinity agent were immobilized. Nucleic acids were

mentioned as bioaffinity agents in this document, which further contemplated a covalent attachment of the coated particles to the support material. Document D1 described these solid support systems as being adaptable for use in several formats (strips, dipsticks, chips, microtiter plates, etc.) and the configuration of the immobilized coated particles on the support material was varied as necessary for the assay format (dot, line, etc.). The document disclosed an embodiment comprising a multi-well device or microtiter plate in which dots of coated particles were placed in the wells on this support material, i.e. beads were directly conjugated to the wells of these plates.

The patent in suit referred to supports of any form, including controlled pore glass beads and cellulose beads and the claims did not require the solid support to have any specific property other than a particular form. These forms were however not characterized in the patent in suit and therefore, there were no limitations associated with the term "*pit*" which was thus equivalent to the term "*well*" as shown by evidence on file.

Document D15 disclosed polyacrolein (PA) microspheres covalently coupled to a solid surface (glass, silicon crystals, polystyrene). Amino ligands were then covalently bound to these immobilized beads. As a solid surface the document disclosed capillaries sealed at both sides which were thus identical in form and size to "*pins*". Except for the "*nanolitre wells*", no volume limitation was associated with the other supports and in particular not for "*an array of pits*". Document D15 disclosed a 96-well ELISA plate (Sero-Well 611F96)

having PA microspheres immobilized on its wells which was thus identical to an array of pits. Although the teachings of document D15 were only exemplified with proteins, reference was made to amino ligands in general and explicitly to the binding of oligonucleotides to immobilized PA surfaces. There was also evidence on file showing that the modification of oligonucleotides with amino groups was known in the art and that amino modified oligonucleotides were available to the skilled person.

First auxiliary request
Article 54 EPC

Document D15 disclosed a microtiter plate, i.e. a two dimensional organized arrangement identical to an array. The more so since no definition of array was found in the patent in suit nor in the claims. In line with the interpretation of "*pit*" made in the main request, a microtiter plate was identical to "*an array of pits*".

Article 56 EPC

In agreement with the case law, the closest prior art was selected among the most promising prior art, namely documents D15 and D1. In particular, document D15 disclosed PA microspheres covalently bound to solid supports including microtiter multi-well plates. These PA microspheres were suitable for binding oligonucleotides and, although this binding required the modification of the oligonucleotides, this modification was routine in the field. Evidence was also on file showing that appropriate modified oligonucleotides were available to the skilled person.

The technical problem to be solved was the provision of alternative solid supports. Document D15 was not limited to microtiter plates but other solid supports (silicon crystals, glass, polystyrene) and forms (pins, glass slides, capillaries) were also contemplated. The substitution of these supports by other supports known in the art, such as arrays of nanolitre wells, did not require any inventive skill.

The patent in suit originally disclosed the technical problem of achieving higher densities of linked nucleic acids and, as a solution, the linking of nucleic acids to beads immobilized in a solid support. The form of the support was not disclosed as contributing to the solution of the problem, since the support was described as being of any desired form, i.e. all forms were equivalent for the purpose of the invention. There was no identification of a particular form or subgroup of forms as providing any advantage. The selection of a subgroup of forms was not directly derivable from the patent nor was it based on a common effect or advantage originally disclosed in the patent. Thus, this selection was arbitrary. In agreement with the case law, any effect based on this selection could not be taken into account for the assessment of inventive step. The contribution of the patent in suit could not be based on a limitation of the subject-matter to a subgroup of forms of the solid support which, as a common feature, had a very small size or volume. This feature was not disclosed as such in the patent in suit let alone as being advantageous, such as providing an increased surface area for binding the oligonucleotides.

XII. The arguments of the respondent in so far as they are relevant to the present decision may be summarized as follows:

Main request
Article 54 EPC

Document D1 disclosed the immobilization of bioaffinity agents onto beads entrapped on a support material. This support was always defined as a flat material (sheet, membrane) without any reference to pits or wells. Nucleic acids were mentioned as possible bioaffinity agents but not exemplified. The coated beads could be covalently attached to the support material yet this was only disclosed as a possible alternative. Document D1 referred to an embodiment comprising a multi-well device or microtiter plate. However, this embodiment did not contemplate the direct attachment of the coated beads to the walls or the bottom of the wells. In fact, it was the flat material with the attached coated beads, i.e. discs of support material with dots of coated particles immobilized thereon, that was placed inside the wells of these microtiter plates.

In the context of the patent, the terms "*pit*" and "*well*" were different and not interchangeable. Whereas the former was understood by the skilled person as a shallow depression or indentation in a surface (not necessarily of a regular and defined shape and thus without a clear bottom and walls), the latter referred to a cavity of a regular and defined shape in a surface (and thus, with bottom and walls). Both terms were present in the claims as granted and thus, could not be objected for lack of clarity.

Although document D15 referred to oligonucleotides, this reference was ambiguous. It was not clear whether it meant the binding of oligonucleotides to the PA microspheres disclosed in the document or else to other surfaces known in the art. In fact, document D15 only disclosed PA microspheres bound to a solid substrate and to amino ligands (proteins). Even if the reference to oligonucleotides was understood in the appellant's sense, it still required to select these oligonucleotides among all other possible amino ligands mentioned in the document. Moreover, direct attachment of oligonucleotides to PA microspheres was not possible without first modifying them. The selection of oligonucleotides as amino ligands did not result inevitably in the claimed subject-matter but required further modification steps. However, document D15 did not indicate how to perform this modification. Therefore, the skilled person was necessarily forced to look for the missing information in the prior art and to perform a further selection among all known methods for modifying the oligonucleotides in a suitable manner. Hence, the disclosure of document D15 taken alone was insufficient for achieving the claimed subject-matter.

Furthermore, the nature and form of the supports of document D15 were different from those of the claims. Since in the context of the patent in suit and for the skilled person the terms "*well*" and "*pit*" were different, a multi-well microtiter plate was not identical to "*an array of pits*". A pin was of a non-hollow nature and thus different from the sealed hollow glass capillaries disclosed in document D15.

First auxiliary request
Article 54 EPC

The multi-well device and the microtiter plates referred to in documents D1 and D15 were different from "*an array of pits*".

Article 56 EPC

According to the case law, the closest prior art was a prior art document aiming at the same objective as the invention. Hindsight of the invention was to be avoided in the selection of this closest prior art. However, hindsight was required for selecting documents D1 or D15 since they did not address the problem of the patent, namely to increase the density of linked nucleic acids. Document D1 intended to achieve a high signal intensity and resist rapid signal fading. Document D15 intended to solve several difficulties known in the art (separation of free and coupled ligands, instability of microspheres suspensions due to agglutination process). This latter document disclosed only proteins as amino ligands, the reference to oligonucleotides being ambiguous. Even if documents D1 and D15 were taken as closest prior art, they did not contain any suggestion leading the skilled person to the problem of the invention let alone to its solution.

The closest prior art was represented by document D25 since it was the sole document on file disclosing DNA arrays and the relevance of the density of linked oligonucleotides. This document referred to the advantages of microparticles and their use as good models in the development of arrays. However, it stated

that microparticles were not amenable to the array format and thus, it actually taught away from the invention. Moreover, there was no indication in document D15 leading the skilled person to combine this disclosure with other prior art and to obtain thereby the claimed arrays. The less so since no other prior art on file related to arrays.

The claimed subject-matter solved the technical problem by providing a system with a high density of nucleic acids per unit of area. In fact, this problem was solved in a two-fold manner, first by coupling nucleic acids to beads and second by immobilizing these coated-beads on supports that had a higher surface area than supports with a flat surface. The claims were limited to a group of non-arbitrarily chosen supports (arrays) that increased the surface area for immobilizing the coated-beads and thereby provided a higher density of linked nucleic acids in addition to that obtained by using beads. In the context of the patent, this group of supports was identified as a preferred embodiment for the purposes of the patent (sequencing by hybridization (SBH) and positional SBH).

XIII. The appellant (opponent) requested that the decision under appeal be set aside and that the patent be revoked.

XIV. The respondent (patentee) requested that the appeal be dismissed (main request) or that the patent be maintained on the basis of the first auxiliary request filed on 11 February 2008.

Reasons for the Decision

Main request
Articles 123(2),(3) and 84 EPC

1. Claim 1 as granted was directed to a composition comprising a bead conjugated to a solid support and to macromolecules defined in granted claim 2 as selected from nucleic acids, peptides, proteins, amino acids and organic molecules. In the opposition proceedings, the composition was limited by the introduction of features of claims 8 and 9 (form of solid support), features taken from the description (type of conjugation) and by restricting the macromolecules to nucleic acids. These limitations, introduced in order to overcome grounds of opposition, narrow the scope of the claims and have a formal support in the application as filed (Articles 123(2),(3) EPC).

2. Claim 1 now refers to a bead conjugated to a solid support, wherein for a particular embodiment the solid support itself is in a form of bead (cf. Section VIII *supra*). The reference in claim 1 and in other claims to "*the bead*" is clearly identifiable as the bead that is to be conjugated to the solid support and not to the bead forming the solid support itself. No ambiguity arises from this wording.

3. No objections have been raised by the opposition division in the contested decision under these articles nor has the appellant raised any objection thereto. The board has no objections of its own.

4. Thus, the main request is considered to fulfil the requirements of Articles 123(2),(3) and 84 EPC.

Article 54 EPC

5. Document D15 discloses the synthesis of surfaces composed of polyacrolein (PA) "*microspheres covalently bound in a monolayer structure onto solid substrates such as glass, silicon crystals, and polystyrene*", which remove some disadvantages associated with the use of polymeric microsphere suspensions (cf. page 258, left-hand column, first and second paragraphs). As suitable surfaces, the document discloses ELISA plates from polystyrene (Sero-Wel 611F96, rigid 96-well plate) and further surfaces in different formats, such as glass slides and glass capillaries, wherein these capillaries are sealed at both sides (cf. page 258, left-hand and right-hand columns, second full paragraphs). These PA microspheres have *"residual aldehyde groups* [*...*] *used for covalent binding of amino ligands, such as proteins*" (cf. page 267, left-hand column, last paragraph), which are the exemplified amino ligands. However, the document also refers to the binding of small molecules, such as haptens and oligonucleotides (cf. page 267, left-hand column, last paragraph).

6. The respondent argues that this reference to oligonucleotides is ambiguous and that the binding of oligonucleotides to PA microspheres requires their modification (for which no indication is given in the document) so as to have amino groups. It further argues that none of the claimed forms for the solid support is

disclosed in document D15 (cf. Section XII *supra*). The board, however, finds these arguments not convincing.

7. Oligonucleotides are explicitly cited in the summary of document D15 and in the context of the advantages of the "*novel modified surfaces composed of PA nanoparticles covalently bound in a monolayer structure onto solid surfaces*", namely "*their high binding strength, high binding stability, high sensitivity, ability to bind small molecules such as haptens and oligonucleotides*" (cf. page 267, left-hand column, last paragraph). No ambiguity arises from this reference which clearly indicates to a skilled person to use oligonucleotides with the surfaces disclosed in the document. Since the residual aldehyde groups of PA microspheres are used for covalently binding the amino ligands, it follows immediately from this reference that amino modified oligonucleotides are required for performing the binding. There is evidence on file showing that this modification is part of the common general knowledge of the skilled person (cf. *inter alia* document D25, paragraph bridging pages 402 and 404, Table 1; in this context see "Case Law of the Boards of Appeal of the EPO", 5th edition 2006, I.C.1.5, page 48). The patent in suit also contemplates the derivatization of nucleic acids and refers to conventional methods as well (cf. paragraphs [0036] and [0037]).

8. According to the established case law, there is no reason to use the description in order to limit the interpretation of a broad claim when assessing its novelty (cf. "Case Law", *supra*, I.C.2.9, page 78). In the present case, although Figure 9 of the patent in suit depicts several pin conformations, neither the

nature or the properties of a pin are defined in the patent in suit nor do they limit in any manner the claimed subject-matter. Similarly, there is no definition of the terms "*well*" and "*pit*" in the patent in suit and therefore, although they might well comprise different entities as argued by the respondent, they certainly comprise an area of overlap (shallow well/deep pit) for which no differences are found. Thus, the sealed glass capillaries and the 96-well plate of document D15 are understood as falling within the terms "*pin*" and "*array of pits*", respectively.

9. The same interpretation applies to the "*multi-well device or microtiter plate*" of document D1 (cf. page 6, line 35). This document discloses "*a bioaffinity agent ... immobilized onto particles or beads which, in turn, are entrapped or immobilized on a porous, absorbent support material*" (cf. *inter alia* page 3, lines 22 and 23), wherein this agent might be an "*appropriate ribonucleic acid or deoxyribonucleic acid nucleic acid*" (cf. page 4, lines 32 to 33 and claim 6). The properties of the particles, the methods for coating these particles and immobilizing them on support material, including a "*permanent covalent attachment*", are described in document D1, which defines the support material onto which the coated particles are immobilized as an "*assay support system*" (cf. page 4, lines 36 to 58 and page 5, lines 2 to 51). The size and shape of the support system are "*selected on the basis of convenience in the selected format*" and "*the configuration of immobilized bioactive agent-coated particles on the support material may be varied as necessary or desirable for the assay format*" (cf. page 5, lines 52 to 59).

10. In the context of the various shapes and configurations of the support systems, document D1 refers to several embodiments, such as an "a*ssay support system ... cut into conveniently sized strips with a dot of coated particles at or near one end*" and a "*support system ... cut into small discs or chips each having a dot of coated particles at or near the center*", wherein "*the coated particles ... are **dotted** onto the support material*" (bold by the board). Immediately thereafter, reference is made to another embodiment, namely *"a multi-well device or microtiter plate in which dots of coated particles are placed in wells on the treated support material*" (cf. page 6, lines 29 to 37). In view of the definitions given for support material and for support system and the indication that coated particles are dotted onto the support material, the board does not share the respondent's interpretation that the whole support system (i.e. coated-beads dotted onto a support material) is placed still on another support (wells of a microtiter plate). On the contrary, the board understands this reference as indicating that the coated particles are directly dotted onto the support material (wells of a microtiter plate) resulting thereby in the assay support system. Moreover, in line with the interpretation given in point 8 *supra*, no differences are seen between a multi-well device and an "*array of pits*".

11. It follows from the above that both documents D1 and D15 anticipate the claimed subject-matter which thus does not fulfil the requirements of Article 54 EPC.

First auxiliary request
Articles 123(2),(3) and 84 EPC

12. The forms of the solid support have been limited to the arrays mentioned in the main request, i.e. "*arrays of pits, arrays of nanolitre wells, beads in an array of pits and beads in an array of nanolitre wells*" (cf. Sections VIII and IX *supra*). No ambiguity arises from this limitation. No objections have been raised by the appellant nor does the board have any of its own. The requirements of Articles 123(2),(3) and 84 EPC are considered to be fulfilled.

Article 54 EPC

13. In view of the interpretation of the terms "*pit*" and "*well*" (cf. point 8 *supra*) and the decision taken on the main request, the respondent announced at the oral proceedings before the board its intention to delete the embodiment "*arrays of pits*" from the first auxiliary request so as to overcome the objection of lack of novelty over documents D1 and D15. However, in view of the board's findings on inventive step (see below), the deletion of this embodiment is not enough for restoring the patentability of this request.

Article 56 EPC

14. In line with the case law, the closest prior art is represented by a prior art document disclosing subject-matter conceived for the same purpose as the claimed invention and having the most relevant technical features in common (cf. "Case Law", *supra*, I.D.3.1, page 121). The board considers that in the

present case document D15 represents the closest prior art.

15. Document D15 refers to the relevance of a high coverage of the support material by PA nanoparticles providing a high concentration of aldehyde groups through which the primary amino ligands are covalently coupled in a single step (cf. page 257, right-hand column, last full paragraph, paragraphs bridging pages 263 and 264 and left-hand and right-hand columns of page 264). The document addresses thereby the same problem as the patent in suit, namely to increase the density of the groups available for coupling to amino ligands and, as a particular group thereof, to oligonucleotides (cf. point 7 *supra*). It further describes the advantages of the disclosed structural arrangement consisting of amino ligand coated PA microspheres attached to the solid support. Although document D15 refers to several supports, none of them corresponds to an array as those mentioned in the request at issue (except for a multi-well plate that, as decided in the main request, is considered to be identical to an "*array of pits*", cf. point 8 *supra*).

16. The respondent argues that the particular group of solid supports of the request at issue has an increased surface area in comparison to other supports and thereby provides a higher density of beads and of linked oligonucleotides (cf. Section XII *supra*). The patent in suit states, however, that "*as compared to "flat" surfaces, beads linked to a solid support provide an increased surface area for immobilization of nucleic acids*" (cf. *inter alia* paragraph [0011] of the patent in suit). There is no reference to increase the

surface area by other means, such as by selecting a particular group of solid supports. In fact, the patent in suit explicitly states that "*the solid support is in any desired form, including but not limited to ... and other geometries and forms known to those of skill in the art*" (cf. paragraphs [0007] and [0038]). Hence, all supports described in the patent are disclosed as being equivalent. The ex-post selection of a particular group of supports in order to address an additional technical problem, namely to further increase the surface area and thereby the density of linked oligonucleotide, finds no support in the patent in suit. Therefore, in line with the established case law, these features cannot be taken into account when formulating the technical problem underlying the invention for the purpose of assessing inventive step (cf. "Case Law", *supra*, I.D.4.4, page 129).

17. Starting from document D15, the technical problem to be solved is thus the provision of alternative supports. The group of arrays of the claimed compositions solves this technical problem.

18. Document D15 discloses several solid supports for immobilizing the coated PA microspheres including ELISA 96-well plates (cf. point 5 *supra*). The use of such a support for immobilizing oligonucleotides coated PA microspheres results in a DNA microtiter plate or a DNA array with microlitre wells. The person skilled in the field of DNA arrays is well aware of the advantages of these plates as well as of their limitations and disadvantages. In particular document D25, a review article on arrays for DNA sequencing and analysis, states that "*owing to the size of the wells and the*

relatively large volume of reagents needed for washing commercially available microtiter plates will probably not become the surface of choice for array hybridizations" (cf. page 404, left-hand column, second full paragraph). However, closely related structural plates with smaller well size and volume, such as plates with nanolitre wells, were well-known to the skilled person and easily available in the art, as the respondent itself acknowledged at the oral proceedings before the board. No particular inventive skill is required for reducing thus the size of the wells of the microtiter plates disclosed in document D15 and select thereby the readily available and closely related structural plates with nanolitre wells referred to in the patent in suit.

19. The claimed subject-matter is thus considered not to fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

A. Wolinski L. Galligani