Internal distribution code:

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

Datasheet for the decision of 24 September 2021

Case Number: T 2042/19 - 3.3.04

Application Number: 13861906.9

Publication Number: 2931950

IPC: C12N15/82

Language of the proceedings: EN

Title of invention:
DNA detection methods for site specific nuclease activity

Applicant:
Dow AgroSciences LLC

Headword:
DNA detection method/DOW AGROSCIENCES

Relevant legal provisions:
EPC Art. 54, 56
RPBA 2020 Art. 13(2)

Keyword:
Main request, auxiliary request 1: novelty - (no);
auxiliary requests 2 to 6: inventive step - (no);
auxiliary requests 7 to 10: admitted - (no).

Decisions cited:

Catchword:

-

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 2042/19 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 24 September 2021

Appellant: (Applicant)	Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268 (US)
Representative:	f & e patent Braunsberger Feld 29 51429 Bergisch Gladbach (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 4 March 2019 refusing European patent application No. 13861906.9 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chair	B. Claes
Members:	R. Morawetz
	R. Romandini

Summary of Facts and Submissions

I. The appeal of the applicant (appellant) lies from the decision of the examining division refusing European patent application No. 13 861 906.9, entitled "*DNA detection methods for site specific nuclease activity*".

II. The following documents are referred to in this decision:

D4 WO 2007/133732
D5 WO 2007/015945

III. The decision under appeal dealt with a main request and six auxiliary requests. The examining division concluded with respect to the main request and auxiliary request 1 that the subject-matter in claim 1 lacked novelty over the disclosure of document D5. With respect to auxiliary request 2, it held that the subject-matter of claim 1 lacked inventive step (Article 56 EPC) when starting from the disclosure in document D5 as the closest prior art. As regards auxiliary requests 3 to 6, it held that the subject-matter of claim 1 lacked inventive step for the same reasons as given for the subject-matter in claim 1 of auxiliary request 2.

IV. With the statement of grounds of appeal, the appellant re-filed the sets of claims of the main request and auxiliary requests 1 to 6.

Claim 1 of the main request reads as follows:

"1. A method for identifying the presence of an exogenous donor DNA polynucleotide inserted within a

targeted genomic locus comprising:
a. amplifying in a first amplification reaction a genomic DNA sample comprising the targeted genomic locus using a first plurality of oligonucleotides that bind under hybridization conditions proximal to the targeted genomic locus, to thereby generate a first amplicon comprising the targeted genomic locus; and,
b. detecting the presence or absence of the first amplicon, wherein the absence of the first amplicon indicates the presence of the exogenous donor DNA polynucleotide within the targeted genomic locus,
the method further comprising:
c. amplifying in a second amplification reaction the genomic DNA sample using a second plurality of oligonucleotides that bind under hybridization conditions proximal to the targeted genomic locus and within the exogenous donor DNA polynucleotide, to thereby generate a second amplicon comprising at least a portion of the targeted genomic locus and at least a portion of the exogenous donor DNA polynucleotide; and,
d. detecting the presence or absence of the second amplicon, wherein the presence of the second amplicon indicates the presence of the exogenous donor DNA polynucleotide within the targeted genomic locus."

Claim 1 of auxiliary request 1 differs from claim 1 of the main request in that the method comprises four additional steps after step d and thus reads as follows (amendments with respect to claim 1 of the main request are highlighted):

" 1. A method for (...) within the targeted genomic locus~~.~~,
the method further comprising:
e. quantitating the results of the first amplification reaction;

f. quantitating the results of the second amplification reaction;
g. comparing the results of the first and second amplification reactions; and,
h. determining the presence or absence of the exogenous donor DNA polynucleotide within the targeted genomic locus, wherein the exogenous donor DNA polynucleotide is confirmed as inserted within the targeted genomic locus if the first amplicon is absent and the second amplicon is present, optionally said method further comprising a multiplex reaction, wherein the first and second amplification reactions are run in a single tube or well."

Claim 1 of auxiliary request 2 differs from claim 1 of the main request in that the term "*subsequently*" is inserted at the end of the expression "*the method further comprising*" between step b and step c.

Claim 1 of auxiliary request 3 is identical to claim 1 of auxiliary request 1 except that step h has been amended. The claim reads as follows (amendments with respect to claim 1 of auxiliary request 1 are underlined):

"1. A method for (...)
h. determining the presence or absence of the exogenous donor DNA polynucleotide within the targeted genomic locus, wherein the exogenous donor DNA polynucleotide is confirmed as inserted within the targeted genomic locus if the first amplicon is absent and the second amplicon is present,
wherein the donor DNA polynucleotide is between 2 and 200 kb in length, optionally between 2 and 10 kb in length,
optionally said method further comprising a multiplex

reaction, wherein the first and second amplification reactions are run in a single tube or well."

Claim 1 of auxiliary request 4 is identical to claim 1 of auxiliary request 3 except that step h has been further amended and claim 1 thus reads as follows (amendments with respect to claim 1 of auxiliary request 3 are underlined):

"1. A method for (...)
h. determining the presence or absence of the exogenous donor DNA polynucleotide within the targeted genomic locus, wherein the exogenous donor DNA polynucleotide is confirmed as inserted within the targeted genomic locus if the first amplicon is absent and the second amplicon is present, wherein the donor DNA polynucleotide is between 2 and 200 kb in length, optionally between 2 and 10 kb in length,
wherein the genomic locus is cleaved by a site specific nuclease,
optionally said method further comprising a multiplex reaction, wherein the first and second amplification reactions are run in a single tube or well."

Claim 1 of auxiliary request 5 is identical to claim 1 of auxiliary request 4 except that step h has been further amended and claim 1 thus reads as follows (amendments with respect to claim 1 of auxiliary request 4 are underlined):

"1. A method for (...)
h. determining the presence or absence of the exogenous donor DNA polynucleotide within the targeted genomic locus, wherein the exogenous donor DNA polynucleotide is confirmed as inserted within the targeted genomic locus if the first amplicon is absent and the second

amplicon is present, wherein the donor DNA polynucleotide is between 2 and 200 kb in length, optionally between 2 and 10 kb in length, wherein the genomic locus is cleaved by a site specific nuclease, wherein said nuclease comprises a zinc finger nuclease, optionally said method further comprising a multiplex reaction, wherein the first and second amplification reactions are run in a single tube or well."

In claim 1 of auxiliary request 6, the amendments made in claim 1 of auxiliary requests 2 and 5 are combined.

V. With a communication dated 26 May 2020, the board summoned the appellant to oral proceedings. In a communication pursuant to Article 15(1) RPBA, the board set out its preliminary opinion with respect to, *inter alia*, claim construction, novelty and inventive step. With respect to inventive step of the subject-matter of claim 1 of auxiliary request 2, the board considered that the disclosure in document D5 was a suitable starting point for assessing inventive step. Based on the effects which could be accepted as being achieved by the claimed invention when compared with the disclosure in document D5, the objective technical problem was defined as the provision of an alternative method for identifying the presence of an exogenous donor DNA polynucleotide inserted within a targeted genomic locus. The board considered that the skilled person would have arrived at the subject-matter of the claim in an obvious manner.

VI. By letter dated 1 July 2021, the appellant submitted sets of claims for auxiliary requests 7 to 10 and arguments in support of inventive step of the newly filed requests. With respect to inventive step of

the subject-matter of claim 1 of auxiliary request 2, the appellant reiterated that the claimed method allowed for a possible reduction in samples to be screened between the two amplification assays.

Claim 1 of auxiliary request 7 reads as follows (amendments with respect to claim 1 of auxiliary request 2 (see section IV) are underlined):

"1. A method for identifying the presence of an exogenous donor DNA polynucleotide inserted within a targeted genomic locus comprising:
a. amplifying in a first amplification reaction a genomic DNA sample comprising the targeted genomic locus using a first plurality of oligonucleotides that bind under hybridization conditions proximal to the targeted genomic locus, to thereby generate a first amplicon comprising the targeted genomic locus; and,
b. detecting the presence or absence of the first amplicon, wherein the absence of the first amplicon indicates the presence of the exogenous donor DNA polynucleotide within the targeted genomic locus,
selecting events from the first amplification reaction which are identified to contain a disrupted genomic locus,
the method further comprising subsequently:
c. amplifying in a second amplification reaction the genomic DNA sample of a selected event using a second plurality of oligonucleotides that bind under hybridization conditions proximal to the targeted genomic locus and within the exogenous donor DNA polynucleotide, to thereby generate a second amplicon comprising at least a portion of the targeted genomic locus and at least a portion of the exogenous donor DNA polynucleotide; and,
d. detecting the presence or absence of the second

amplicon, wherein the presence of the second amplicon indicates the presence of the exogenous donor DNA polynucleotide within the targeted genomic locus."

Claim 1 of auxiliary request 8 differs from claim 1 of auxiliary request 7 in that in line 1 the expression "*high throughput*" is inserted before the term "*method*".

Claim 1 of auxiliary request 9 differs from claim 1 of auxiliary request 8 in that after step d, the following expression is inserted: "*wherein the first amplification reaction and subsequent detection represents a disruption assay and the second amplification reaction and subsequent detection represents an In-Out PCR assay.*"

Claim 1 of auxiliary request 10 differs from claim 1 of auxiliary request 2 in that after step d, the following steps are inserted so that claim 1 reads as follows (amendments with respect to claim 1 of auxiliary request 2 are highlighted):

" 1. A method for (...) within the targeted genomic locus~~.~~,
the method further comprising:
e. quantitating the results of the first amplification reaction;
f quantitating the results of the second amplification reaction;
g. comparing the results of the first and second amplification reactions, and,
h. determining the presence or absence of the exogenous donor DNA polynucleotide within the targeted genomic locus, wherein the exogenous donor DNA polynucleotide is confirmed as inserted within the targeted genomic

locus if the first amplicon is absent and the second amplicon is present;
the method further comprising selecting only samples for which the first amplicon is absent and the second amplicon is present for further use".

VII. With the consent of the appellant, the oral proceedings were held by videoconference. At the end of the oral proceedings, the Chair announced the board's decision.

VIII. The appellant's arguments are summarised as follows.

Main request - claim 1

Claim construction

The use of the indefinite article ("a genomic DNA") in step a and the use of the definite article ("the genomic DNA") in step c established a particular order of the amplification steps, namely that steps a and b were performed before steps c and d.

A targeted genomic locus allowed the design of primers and probes for the integration site.

Novelty (Article 54(2) EPC)

The claimed subject-matter was novel over the disclosure of document D5 as this document disclosed neither (i) the sequential order of the disruption amplification assay and the event-specific PCR (or In-Out PCR) assay as claimed nor (ii) targeted insertion. In contrast, document D5 referred to event 40-3-2, which was a random integration event.

Inventive step (Article 56 EPC)

Closest prior art

The disclosure in document D4, not D5, represented the closest prior art. Document D4 related to a method for identifying a targeted integration, while document D5 related to a method for determining the zygosity of a hybrid progeny and was silent about a method for identifying the presence or absence of an insert inserted within a targeted genomic locus.

Also starting from the disclosure in document D5 as the closest prior art, the claimed subject-matter was inventive.

Objective technical problem

The primary difference was that the claimed subject-matter was an assay to identify the donor insertion within a known (targeted) genomic locus, whereas document D5 disclosed an event assay of a randomly inserted gene expression cassette within the plant genome. Targeted insertion provided a technical advantage not offered by document D5 because with targeted insertion, the person skilled in the art could design primers and probes for the genomic location at which the insertion occurred.

Further differences between the subject-matter of claim 1 and the disclosure in document D5 were the sequential order of the amplification reactions and that the claim was directed to the high-throughput screening of a large number of events.

The effects resulting from these further differences were that the user had the opportunity to perform the first amplification reaction with a positive control and that the number of samples for the second reaction could be reduced.

No objective technical problem was formulated.

Obviousness of the claimed solution

Document D5 provided no reason to change the order of the amplification reactions.

It was not obvious for a person skilled in the art to perform the claimed method with the disruption assay in the first place and the event-specific amplification in the second place.

Auxiliary request 1 - claim 1

Novelty (Article 54(2) EPC)

The subject-matter was novel for the same reasons as given for claim 1 of the main request.

Auxiliary request 2 - claim 1

Novelty (Article 54(2) EPC)

The subject-matter was novel because in document D5 the event-specific amplification corresponding to steps c and d was performed first (see paragraph [0011]).

Inventive step (Article 56 EPC)

The subject-matter was inventive for the same reasons as given for claim 1 of the main request. In addition, in document D5, the results of both assays were needed to deduce whether the event was absent from both alleles, whereas the claimed method allowed for a possible reduction in samples to be screened between the two amplification assays.

Auxiliary requests 3 to 7 - claim 1

Inventive step (Article 56 EPC)

The subject-matter was inventive for the same reasons as given for claim 1 of the main request.

Auxiliary requests 7 to 9

Admission (Article 13(2) RPBA 2020)

The filing of these requests was a direct reaction to the board's preliminary opinion that claim 1 of auxiliary request 2 did not recite the technical features responsible for the technical effect relied on in support of the inventive step argument. Stating that the claim lacked these technical features was a new objection.

IX. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the set of claims of the main request or, alternatively, on the basis of the set of claims of one of auxiliary requests 1 to 6, all these requests underlying the decision under appeal and re-submitted with the statement of grounds of appeal or,

alternatively, on the basis of the set of claims of one of auxiliary requests 7 to 10, all these requests submitted with the letter dated 1 July 2021.

Reasons for the Decision

1. The appeal complies with Articles 106 to 108 and Rule 99 EPC and is admissible.

Main request - claim 1

Claim construction

2. The claim relates to a method for identifying the presence of an "*exogenous donor DNA polynucleotide*" inserted within a "*targeted genomic locus*". According to the wording of the claim, the method comprises two amplification reactions: the first is the disruption amplification assay specified in steps a and b; the second is the In-Out event-specific amplification assay specified in steps c and d.

3. The skilled person reading the claim infers from the use of the indefinite article for the genomic DNA in step 1 a, "*a genomic DNA sample*", followed by use of the definite article in step 1 c, "*the genomic DNA sample*", that the same template, i.e. a genomic DNA sample comprising the targeted genomic locus, is used in steps a and c. However, the skilled person would not infer any sequential or temporal order of the two amplification reactions from the wording of the claim.

4. With respect to the "*targeted genomic locus*", the board considers that "*targeted*" is a product-by-process feature which in the circumstances of the claimed method is understood to specify the locus in which the

insertion of the exogenous DNA takes place. The targeting does not result in a distinct and identifiable characteristic which would distinguish the genomic locus from any other kind of genomic locus. It merely implies that the genomic locus at which the insertion has taken place is known such that oligonucleotides that bind proximal to it can be designed and used as primers in a PCR amplification process.

Novelty (Article 54(2) EPC)

5. Document D5 discloses a method of determining the presence of a transgene, i.e. an exogenous donor DNA polynucleotide, inserted within a particular region in the soybean genome, i.e. a targeted genomic locus (see paragraph [0011] and Figure 1). The method comprises two amplification reactions. The first uses a primer set that produces a first amplicon which is diagnostic for the transgenic event, i.e. an event-specific In-Out amplification assay (see page 4, lines 1 to 7 and Figure 1). The second amplification reaction uses a primer set that produces a second amplicon comprising the native genomic DNA, with the presence of the second amplicon indicating the absence of the transgene and the absence of the second amplicon indicating the presence of the transgene, i.e. a disruption amplification assay (see page 4, lines 7 to 13 and Figure 1). The first amplification reaction aims at confirming the insertion of a transgene in the region of interest, while the second amplification reaction aims at detecting the presence or absence of the transgene.

6. The appellant submitted that the claimed subject-matter was novel over the disclosure of document D5. In its

view, this document disclosed neither (i) the particular order of the disruption assay and the event-specific PCR assay as claimed nor (ii) targeted insertion/integration but rather random integration.

7. However, as set out in points 3. and 4. on claim construction, the claim does not prescribe a particular order of the two amplification assays. Moreover, it is immediately apparent to the skilled person that the method of document D5 is based on the knowledge of the genomic locus where the transgene is inserted since this is a prerequisite for the design of the primers used in the method of document D5 (see paragraph [0011], Figure 1 and Table 1). Hence, in document D5, the insertion takes place at a known site and is thus also "*targeted*". In this context, the board notes that whether soybean event 40-3-2 as disclosed in document D5 is the result of random integration is irrelevant since the method of document D5 is based on the knowledge of the insertion site of the 40-3-2 event and aims at the determination of the presence or absence of the insert in the progeny plants (see e.g. Figure 1).

8. The board concludes from the above considerations, in agreement with the decision under appeal, that the claimed subject-matter is not novel over document D5.

Auxiliary request 1 - claim 1

Novelty (Article 54(2) EPC)

9. In this context, the appellant relied on its argument submitted with respect to claim 1 of the main request. This argument is not found persuasive by the board (see points 6. and 7.). Therefore, the board sees no reason

to set aside the decision under appeal on the basis of this argument.

Auxiliary request 2 - claim 1

Novelty (Article 54(2) EPC)

10. The claim specifies that the In-Out event-specific amplification assay specified in steps c and d is performed after the disruption amplification assay specified in steps a and b. The order of the two amplification assays thus differs with respect to the order disclosed in document D5 (see point 5. above).

11. The claimed subject-matter is thus novel over the disclosure in document D5.

Inventive step (Article 56 EPC)

Closest prior art

12. In the decision under appeal, the disclosure of a method for identifying the presence of an exogenous DNA at a known locus in document D5 was held to represent the prior art closest to the claimed method. This was contested by the appellant, which considered the disclosure in document D4 to represent the closest prior art.

13. The closest prior art is normally a document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common (see Case Law of the Boards of Appeal, 9th edition 2019, "CLBA", section I.D.3.1).

14. Here, the claimed invention aims at identifying the presence of an exogenous donor DNA polynucleotide inserted within a targeted, i.e. known, genomic locus. Contrary to the submission by the appellant, document D5 shares this objective (see point 5.) and the most relevant technical features with claim 1 (*ibid.*). Hence, the disclosure in document D5 is a suitable starting point for the assessment of inventive step.

15. If the skilled person has a choice of several workable routes starting from different disclosures suitable to represent the closest prior art and if the invention is obvious in respect of at least one of these routes, as is the case here (see point 16. ff below), an inventive step is lacking (see CLBA, section I.D.3.1). Accordingly, the appellant's line of argument based on document D4 as representing the closest prior art need not be considered.

Objective technical problem

16. The claimed subject-matter differs from the disclosure in document D5 in the order of the two amplification steps, while the purpose of the two amplification steps is the same as in document D5 (see point 5.).

17. With respect to the effects caused by this difference, the appellant submitted that in a high-throughput assay, the first series of PCR reactions were necessary to overcome the detection of false positives that would occur if only the second series of PCR reactions was performed. By performing the disruption assay first, a positive control could be included, while in an event-specific amplification, no positive control was possible. Furthermore, the claimed method allowed for a

possible reduction of the samples for the second reaction. The "primary difference" was that the method allowed for the identification of an insertion within a known genomic locus.

18. However, the claimed subject-matter is not restricted to high-throughput assays, no controls are included, the samples are not reduced for the second amplification assay and the two amplification assays are carried out entirely independent of each other. Indeed, the claim specifies that the method comprises both amplification assays, and no selection of samples based on the results of the first amplification assay is specified. In other words, the appellant relies on effects caused by technical features not specified by the claimed subject-matter. Moreover, the method of document D5 allows, in the board's view, for the identification of an insertion within a known genomic locus (see point 5.). Hence, the board is not convinced that the "primary difference" relied on by the appellant exists.

19. Accordingly, the claimed invention does not achieve a technical effect which goes beyond the one obtained by the method disclosed in document D5.

20. The objective technical problem to be solved by the claimed subject-matter is the provision of an alternative method for identifying the presence of an exogenous donor DNA polynucleotide inserted within a targeted genomic locus.

Obviousness of the claimed solution

21. The question to be answered in assessing obviousness is whether the skilled person seeking to solve the problem

formulated above and starting from the method disclosed in document D5 would have modified that method to arrive at the claimed invention in an obvious manner.

22. In the board's judgement, the skilled person reading document D5 would have realised that both amplification assays need to be performed to identify the presence of an exogenous donor DNA polynucleotide inserted within a targeted genomic locus. They would furthermore have realised that these assays are entirely independent and can thus be carried out in any order.

23. The skilled person, when faced with the technical problem, would have readily considered changing the order of the two amplification steps and, hence, have arrived in an obvious manner at the claimed subject-matter.

24. In view of the above considerations, in agreement with the decision under appeal, the claimed subject-matter does not involve an inventive step (Article 56 EPC).

Auxiliary requests 3 to 7 - claim 1

Inventive step (Article 56 EPC)

25. In this context, the appellant relied on its line of argument submitted for inventive step of the subject-matter of claim 1 of the main request but did not provide any further arguments tailored to these requests. The appellant's arguments are dealt with in the context of auxiliary request 2 and are not found persuasive (see points 12. to 23.). Therefore, the board sees also no reason to set aside the decision under appeal on the basis of this argument. *Auxiliary*

requests 7 to 10

Admission (Article 13(2) RPBA 2020)

26. These claim requests were filed in response to the board's communication in which it was held, *inter alia*, that the skilled person would have arrived in an obvious manner at the subject-matter of claim 1 of auxiliary request 2.

27. A new version of the Rules of Procedure of the Boards of Appeal (RPBA 2020; "RPBA"; OJ EPO 2020, Supplementary publication no. 1, III.2) entered into force on 1 January 2020. The transitional provisions are set out in Article 25 RPBA. In the case at hand, the summons to oral proceedings was notified after 1 January 2020 (see section V.). Therefore, Article 13(2) RPBA governs the admittance of auxiliary requests 7 to 10.

28. Claim 1 of auxiliary requests 7 to 10 was amended to include further features such as, *inter alia*, a selection step between the two amplification assays, an indication that the method is a high-throughput method and the addition of a selection step after performance of the two amplification assays (see section VI.). These features are all taken from the description of the application as filed. Their insertion in claim 1 results in subject-matter not pursued so far in the appeal proceedings and represents an amendment of the appellant's case.

29. Pursuant to Article 13(2) RPBA 2020, any amendment to a party's appeal case after notification of a summons to oral proceedings must, in principle, not be taken into account unless there are exceptional circumstances

which have been justified with cogent reasons by the party concerned.

30. In the case at issue, lack of inventive step of the subject-matter of claim 1 of auxiliary request 2 had been among the grounds for refusal in the decision under appeal (see section III.). In the communication setting out its preliminary opinion, the board defined the objective technical problem on the basis of effects which could be accepted as being achieved by the claimed invention when compared with the disclosure in document D5 (see section V.).

31. The fact that the board has defined the objective technical problem on the basis of the technical effect(s) achieved by the subject-matter as defined in the claim does not qualify as a new objection raised by the board. Rather, it is a consequence of the application of the problem and solution approach in assessing inventive step (see CLBA, section I.D.2.).

32. The board could thus not see how the fact that the board was not persuaded by the appellant's arguments with respect to inventive step of the subject-matter of claim 1 of auxiliary request 2 can qualify as exceptional circumstances within the meaning of Article 13(2) RPBA 2020.

33. As a consequence, the board did not admit auxiliary requests 7 to 10 in the appeal proceedings (Article 13(2) RPBA 2020).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

I. Aperribay B. Claes

Decision electronically authenticated