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Datasheet for the decision of 26 January 2017

Case Number: T 1607/13 - 3.3.07

Application Number: 08153863.9

Publication Number: 1952792

IPC: A61K6/00, C12Q1/68

Language of the proceedings: EN

Title of invention:
Methods of using FET labeled oligonucleotides that include a 3' 5' exonuclease resistant quencher domain and compositions for practicing the same

Patent Proprietor:
Life Technologies Corporation

Opponents:
Adams, Harvey Vaughan John

Headword:
Methods of using FET labeled oligonucleotides that include a 3' 5' exonuclease resistant quencher domain and compositions for practicing the same/Life Technologies Corporation

Relevant legal provisions:
EPC Art. 123(2), 76(1), 100(b), 84, 56

Keyword:
Main Request - Amendments (no)
Auxiliary request 1 - Amendments (yes)
Auxiliary request 1 - Lack of disclosure and lack of clarity (no)
Auxiliary request 1 -Inventive step (yes)

Decisions cited:
G 0003/14

Catchword:

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Case Number: T 1607/13 - 3.3.07

D E C I S I O N
of Technical Board of Appeal 3.3.07
of 26 January 2017

Appellant: (Patent Proprietor)	Life Technologies Corporation 5823 Newton Drive Carlsbad, CA 92008 (US)
Representative:	Roques, Sarah Elizabeth J A Kemp 14 South Square Gray's Inn London WC1R 5JJ (GB)
Appellant: (Opponent 1)	Adams, Harvey Vaughan John Mathys & Squire LLP 120 Holborn London EC1N 2SQ (GB)
Representative:	Wise, Daniel Joseph Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)

Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 8 May 2013 concerning maintenance of the European Patent No. 1952792 in amended form.**

Composition of the Board:

Chairman	A. Usuelli
Members:	D. Boulois
	P. Schmitz

Summary of Facts and Submissions

I. European patent No. 1 952 792 was granted on the basis of a set of 18 claims.

Independent claim 1 and 11 as granted read as follows:

"1. A FET labeled probe comprising a nucleic acid intercalator which comprises a polycyclic compound bonded to a FET labeled oligonucleotide, wherein said nucleic acid intercalator is covalently bonded to the 3' end of said FET labeled oligonucleotide and wherein said FET labeled oligonucleotide comprises a dark quencher located at its 3' end and further wherein said FET labeled probe is 3'->5' exonuclease resistant."

"11. A kit for use in detecting the production of a primer extension product in a primer extension reaction mixture, said kit comprising: (a) a FET labeled oligonucleotide that includes a 3'->5' exonuclease resistant quencher domain and a nucleic acid intercalator covalently bonded to its 3' end, wherein the FET labelled oligonucleotide includes a dark quencher located at its 3' end, and further wherein the intercalator comprises a polycyclic compound."

II. The patent was opposed under Article 100(a), (b) and (c) EPC, on the grounds that its subject-matter lacked novelty and inventive step, was not sufficiently disclosed and extended beyond the content of the application as filed.

III. The appeals by the patent proprietor and opponent (hereinafter respectively referred to as the appellant-proprietor and the appellant-opponent) lie from the decision of the opposition division to maintain the

patent as amended. The decision was based on the sets of claims as granted, as main request and auxiliary request 1 as filed during the oral proceedings on 27 February 2013.

IV. The documents cited during the opposition proceedings included the following:
D1: WO01/86001
D2: WO00/41549
D6: US 5 177 196
D7: Goodchild J., Bioconjugate Chem., vol. 1, no. 3., p.165-187
D21: Kutyavin et al, Nucleic Acids Research, vol. 28, no. 2, p655-661
D23: W099/51621
D24: WO01/42505
D33: US 5 419 966
D36: Dearing el al, Nucleic Acids Research, vol. 9, no. 6, p 1483-1497
D41: Asseline et al, Bioconjugate Chem., 7, 1996, 369-379
D45: Wikipedia extract "Intercalation".

V. According to the decision under appeal, the feature in claims 1 and 11 that the intercalator "comprises a polycyclic compound" found a basis in originally filed claims 1 and 6 of the patent in suit (Article 123(2) EPC), but there was no corresponding basis in the parent application as filed, contrary to the requirements of Article 76(1) EPC. Furthermore, the originally filed claims of the patent in suit provided no basis for this feature in the context of the claimed kit, so that the presence of this feature in claim 11 also contravened Article 123(2) EPC.

The feature in claims 1 and 11 “a dark quencher is located at its 3' end” did not find a basis in the parent application or the original application documents of the patent in suit (Articles 76(1) and 123(2) EPC), since the description did not use the terms "3' end" and "3' terminus" in an interchangeable manner.

Dependent claims 3 and 4 expressed embodiments of claim 1 wherein the intercalator provided increased stability to the hybrid formed from the FET-labelled oligonucleotide, and exonuclease resistance to the FET-labelled oligonucleotide respectively. These claims corresponded to originally filed claims 4 and 5, and therefore had a basis under Article 123(2) EPC. No corresponding claims were however present in the parent application and the relevant part of the description on page 15, l.17-23, provided a different teaching to the skilled person, thereby giving rise to added subject-matter, in contravention of Article 76(1) EPC.

Similar considerations applied to claims 10 and 15, which represented added subject-matter compared with the parent application (Article 76(1) EPC).

In conclusion, the main request did not meet the requirements of Articles 76(1) and 123(2) EPC.

The auxiliary request filed during oral proceedings was considered admissible and met the requirements of Articles 76(1) and 123(2) EPC.

The subject-matter of the auxiliary request differed from the main request in specifying that the nucleic acid intercalator "**is** a polycyclic compound" in independent claims 1 and 9 (previously independent

claim 11), and by deleting dependent claims 3, 4, 15 and some features of dependent claim 8 (previously dependent claim 10).

The subject-matter of the auxiliary request was found to be novel.

As to inventive step, D1 was considered to represent the closest prior art, and claim 1 of the auxiliary request differed from the BH1 probe of D1 in that the probe comprised a nucleic acid intercalator, which was a polycyclic compound covalently bonded to the 3' end of the FET-labelled oligonucleotide.
The technical effect that resulted from this difference was indicated by example 6 of the patent, which showed that a 3' terminal acridine on the FET-labelled probes provided reduced noise and a more accurate result than with a 5' terminal. The technical problem to be solved over D1 was the provision of a FET-labelled oligonucleotide detection probe showing improved signal to noise ratio in a real-time PCR assay.
The solution, namely to bind covalently a polycyclic intercalator at the 3' end, was considered to involve an inventive step, since there was no prompting from the prior art to modify the BH1 probe of D1 to arrive at a probe as in claim 1.

The claimed invention was also considered to be sufficiently disclosed.

VI. With its statement setting out the grounds of appeal dated 11 September 2013, the appellant-proprietor requested that the patent be maintained as granted and filed 2 auxiliary requests.

VII. With its statement setting out the grounds of appeal dated 18 September 2013, the appellant-opponent filed *inter alia* following documents:
D52: Declaration on behalf of Professor Tom Brown, University of Oxford including: Annex A (Curriculum Vitae of Professor Tom Brown)
Annex B (Ranasinghe etal. 2001, Chem. Commun., 2001:1480-1481)
Annex C (Extract from Glen Research Product Catalog)
Annex D (Thuong et al. 1987, PNAS, 84:5129-5133)
Annex E (Nelson et al. 1992, Nucleic Acids Research, 20(23):6253-6529)
Annex F (Asseline et al. 1984, The EMBO Journal, 3(4): 795-800)
Annex G (Asseline & Thuong, 1997, New J. Chem., 21:5-17)
Annex H (Helene, 1989, Br. J. Cancer, 60:157-160)
Annex I (Asseline et al. 1984, PNAS, 81:3297-3301)
Annex J (Toulme et al. 1986, PNAS, 83:1227-1231)
Annex K (Lancelot et al. 1985, Biochemistry, 24:2521-2529).

VIII. With a letter dated 4 February 2014, the appellant-proprietor filed a third auxiliary request.

IX. A communication from the Board, dated 10 January 2017, was sent to the parties.

X. With a letter dated 16 January 2017, the appellant-proprietor submitted new auxiliary requests 1 to 4.

In auxiliary request 1, the subject-matter of independent claims 1 and 11 has been amended by specifying that the nucleic acid intercalator "**is** a polycyclic compound".

Additionally, dependent claim 10 was amended and claim 15 was deleted.

XI. Oral proceedings took place on 26 January 2017.

XII. The arguments of the appellant-opponent may be summarised as follows:

Added matter

Granted claims 1, 3, 4 ,10, 11 and 15 contained added subject-matter contravening Articles 76(1) and/or 123(2) EPC. The features objected were the following:

a) **The wording of claim 1**

From a sensible reading of the claim, the FET-labelled probe comprised a FET-labelled oligonucleotide and an intercalator bonded to the 3' end of the FET-labelled oligonucleotide, and in addition it had a dark quencher located at the 3' end of the FET-labelled oligonucleotide; the claimed probe might therefore contain two quenchers. There was no basis for such an interpretation in the original application.

b) Feature **"the nucleic acid intercalator comprises a polycyclic compound"** in claims 1 and 11

There was no basis on page 15 or anywhere else in the application as filed for "a nucleic acid intercalator comprising a polycyclic compound", but only for a nucleic acid intercalator being/consisting of a polycyclic compound. Claims 1 and 11 thus contravened Articles 76(1) and 123(2) EPC.

c) Feature **"wherein the dark quencher is located at the 3' end"** in claims 1 and 11

The disclosures of "3' end" in the context of the methods, systems and kits in the claims and in the description could not be used as a pool to combine with other generic disclosures appearing in the description in order to arrive at a specific embodiment that was not originally envisaged. As such, the subject-matter of claims 1 and 11 contravened Articles 76(1) and 123(2) EPC.

d) Feature **"dark quencher"** in claims 1 and 11

With reference to lines 10-13 on page 12, the disclosure of the acceptor being a dark quencher was made in the context of imparting 3 -5' exonuclease resistance onto the FET-labeled oligonucleotide. Thus, the patentee had generalised from the disclosure in the application as filed. As such, the subject-matter of claims 1 and 11 contravened Articles 76(1) and 123(2) EPC.

e) The subject-matter of **claims 3, 4 and 15**

Claim 3 was dependent on claim 1 and recited that the nucleic acid intercalator provided increased stability to the hybrid formed from the FET-labelled oligonucleotide. This meant that claim 1 encompassed a FET-labelled probe having the structural features recited in claim 1 but where the nucleic acid intercalator did not provide increased stability to the hybrid formed from the FET-labelled oligonucleotide e.g. due to the presence of some other component.

Claim 4 was dependent on claim 1 and recited that the nucleic acid intercalator provided exonuclease activity resistance to the FET-labelled oligonucleotide. This meant that claim 1 encompassed a FET-labelled probe having the structural features recited in claim 1 but where the nucleic acid intercalator did not provide exonuclease activity resistance to the FET-labelled oligonucleotide, e.g. where the exonuclease activity resistance of the FET-labelled probe was due to the presence of some other component.

Claim 15 recited that the minor groove binder provided increased stability to the hybrid formed from the FET-labelled oligonucleotide. This meant that claim 11 encompassed a kit where the recited minor groove binder did not provide increased stability to the hybrid formed from the FET-labelled oligonucleotide. Such a kit was not disclosed on page 16 or anywhere else in the application as filed.

The provisions of Article 76(1) EPC were therefore clearly contravened.

f) Deletion of claims 3, 4 and 15 in the auxiliary requests

The appellant-opponent noted further that claims 3, 4 and 15 had been deleted in the Patentee's auxiliary requests. However, infringement of Article 76(1) EPC could not be overcome by deleting of these dependent claims. The problem under Article 76(1) EPC arose because, when formulating granted claims 1 and 11 and 13, the patentee had failed to include the functional features of the disclosures concerning the intercalator and minor groove binder (see point e) above). This could only be rectified by amending granted claims 1

and 11 to include the functional features of the intercalator and by amending granted claim 13 to include the functional feature of the minor groove binder.

g) The subject-matter of **claim 10**

There was no basis in the application as filed for *in situ* hybridisation, thus contravening Article 76(1) EPC.

Inventive step

The closest prior art was D1 which disclosed the following sequences:
5'FAM-oligonucleotide-BH1 3'
5'FAM-oligonucleotide-DABCYL 3'.

The difference with the claimed probe was the absence of a 3' end intercalator. Starting from these probes, the technical problem to be solved might be defined as to develop these probes to enhance mismatch discrimination when used in real-time PCR.

The alleged technical problem was already taught in the prior art, and the data in Example 6 did not provide any evidence of a technical effect.

There were no data in Example 6 to support a technical effect for the claimed probe:
a) The results in Table 7 of Example 6 could not assist in demonstrating the alleged effect, since no results were provided for a 5'ACR probe.
b) The probes used in Example 6 did not fall within the scope of the claims. The ACR was acting as a linker between the BHQ1 and the oligonucleotide.

c) There was no evidence whatsoever that the 3'ACR in the exemplified probes was intercalating. Since it was positioned at the 3' terminus, it was not known whether the effect allegedly observed was due to intercalation or by participation of the ACR in Π-stacking.
d) There was another explanation as to why different results were obtained for 5' ACR and 3' ACR probes. The 5' digestion of the 3'-ACR-BHQ probe by the polymerase left the 5'-TET free of nearby quencher and able to strongly fluoresce, whereas in the 5'-TET-ACR case the TAE and ACR probably remained together (see D52).
e) The claims of each requests were too broad in comparison to the exemplified probes of Example 6. The alleged effect could not be extended beyond the specific probe shown.
f) Moreover, the data of Example 6 did not support the technical effect of allele discrimination. The data of the example were not provided in the context of a competitive setting. The data could not support an alleged advantage of improved signal-to-noise ratio and of improving allele discrimination in high fidelity, real-time PCR, since the tests used a single probe and a single template in each experiment, whereas they should have been performed in a competitive setting.

When faced with said technical problem, the skilled person would anyway have attached an intercalator as a matter of routine design choice. An intercalator stabilised fully complementary duplexes more than mismatched complexes, as explained in D52. Annex B of D52 further confirmed that the effect of enhanced mismatch discrimination could be achieved if the intercalator was positioned away from the 3' terminus of the probe. The position of the intercalator at the 3' end represented nothing more than an arbitrary modification with no unexpected effect. The skilled

person had in any case a 50:50 chance of choosing the 3' end on the basis of paragraph [0026] of the patent which confirmed that "the 3' end means at any location on the oligonucleotide from and including the 3' terminus to the center of the oligonucleotide".

D1 also specifically mentioned on page 41 that the probes could be modified with an intercalating agent. Documents D2, D6, D7, D33 and D41 disclosed the teaching regarding the technical effect of a 3' terminus intercalator. D50 also showed that minor groove binders and intercalators were interchangeable.

Thus, the claimed subject-matter lacked inventive step starting from the probes of D1 when modified with an intercalator as mentioned in D1, D2, D6, D7, D33, D41 and D50.

Document D24 was also considered to represent an alternative starting point for the assessment of inventive step. This document disclosed a FET oligonucleotide with a 5' fluorophore, a 3' dark quencher and a minor groove bonder (MGB) linked to the 3' end. This document had been considered in relation to inventive step, but dismissed by the opposition division, because intercalators and minor groove binders were not interchangeable, a point on which the opposition division had erred.
D2 taught that intercalators and minor groove binders belonged to the class of "hybridisation stabilising moieties" and were functionally interchangeable. The solution was therefore obvious.

Insufficiency of disclosure

In view of the narrow exemplification of the claimed subject-matter, the skilled person was not provided with enough information to make probes that worked across the full scope of the claim.

XIII. The arguments of the appellant-proprietor may be summarised as follows:

Added matter

a) **The wording of claim 1**

Claim 1 did not permit several possible interpretations. The reading proposed by the appellant-opponent was based on an artificial analysis.

b) Feature **"the nucleic acid intercalator comprises a polycyclic compound"** in claims 1 and 11

A basis for this feature was to be found in claim 1 in combination with claim 6 of the application as filed, and on page 15 of the parent application.

c) Feature **"wherein the dark quencher is located at the 3'end"** in claims 1 and 11

A basis for this features could be found in claims 10, 14, 37, 38, 54 and 55 of the parent application and in corresponding paragraphs of the application as filed.

d) The subject-matter of **claims 3, 4 and 15**

The objection raised by the opponent was for lack of clarity, not for added subject-matter. A basis for these claims could however be found on pages 15 and 16 of the original application.

e) The subject-matter of **claim 10**

This disclosure was implicit in the application, and was referred to on page 8, lines 1-3 of the original description, and on page 13, line 30.

Inventive step

The invention was directed to the provision of a FET-labeled probe with improved signal-to-noise ratio. The problem over D1 could be considered as how to further improve the signal-to-noise ratio. The solution was the presence of a 3' intercalating agent.

In Example 6 of the patent, studies had been conducted to assess allele discrimination using acridine labeled FET probes under high-fidelity PCR, and this example showed clearly that the cross-reactivity or noise was reduced when using 3' ACR labeled probes.

As to the obviousness of the solution, none of the cited documents was relevant:
- D1 referred to black hole quenchers and did not specify whether it could be provided at the 3' or 5' end
- the probes described in D2 were different from that of the contested patent, and intercalators were mentioned as hybridization stabilising moieties
- the intercalators were used in D6 to enhance binding affinity, and there was no suggestion to use such intercalators in a FET probe, let alone for solving the problem posed
- D7 related to the chemical synthesis of modified oligonucleotides and their properties

- with reference to D24, the opponent sought to argue that intercalators and minor groove binders were interchangeable. It was however clear that there were different types of compound as shown by many other cited documents
- D33 was concerned with the provision of 3' tailed oligonucleotides, and highlighted the benefits of such modified oligonucleotides in the presence of serum
- D41 was concerned with synthetic oligonucleotides for antisense uses.

As to Professor Brown's declaration D52, no evidence had been provided to support the assertion that there was no position effect associated with the introduction of an intercalator. The reference to Annex B of D52 was not concerned with a FET oligonucleotide probe having a quencher molecule at the 3' terminus and additionally having a 3' intercalator. The analysis of melting temperatures conducted in Annex B did not provide a comparison of probes having acridine in different positions. Thus, Annex B did not demonstrate that there was no difference depending on the position of acridine.

As regards D24 as closest prior art, this document did not disclose the presence of an intercalating agent, but only of a minor groove binder, whose function was different, since it improved the binding and discrimination in PCR assays (see D24, page 10, l. 18-30), as also shown by D23. The two types of molecules were not interchangeable, and intercalators were not known as having this property.

Insufficiency of disclosure

No evidence had been put forward by the opponent to support the assertion that a person skilled in the art could not produce the claimed probes.

XIV. **Requests**

The appellant-proprietor requested that the decision under appeal be set aside and that the opposition be rejected, i.e. that the patent be maintained as granted, alternatively that the patent be maintained on the basis of one of auxiliary requests 1 to 4 filed with letter dated 16 January 2017.

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

Reasons for the Decision

1. Main request - Amendments (Article 100(c) EPC)

According to the appellant-opponent, claims 1, 3, 4, 10, 11 and 15 as granted contravene Articles 76(1) and/or 123(2) EPC.

1.1 Article 123(2) EPC

1.1.1 **Claims 1 and 11**

The whole wording of claim 1 was objected to, as well as the features **"the nucleic acid intercalator comprises a polycyclic compound"**, **"dark quencher"** and **"wherein the dark quencher is located at the 3'end"** in claims 1 and 11.

The subject-matter of claim 1 as granted is based on claim 1 of the application as filed which reads as follows:
"1. A FET labeled probe comprising a nucleic acid intercalator bonded to a FET labeled oligonucleotide, wherein said nucleic acid intercalator is covalently bonded to the 3' end of said FET labeled oligonucleotide."

This subject-matter of original claim 1 was supplemented by the following features:
i) "which comprises a polycyclic compound"
ii) "and wherein said FET labeled oligonucleotide comprises a dark quencher located at its 3' end"
iii) "and further wherein said FET labeled probe is 3'->5' exonuclease resistant."

Features i) and iii) originate directly and unambiguously from original claims 6 and 2 of the application as filed.

Feature ii) is to be found in several parts of the description as originally filed, relating directly to said FET-labeled probe involved in a method of PCR amplification, or a system or kit comprising said FET-labeled probe, such as:
- on page 38, lines 6-7:
"wherein said dark quencher is located at the 3' end of said FET labeled oligonucleotide".
- on page 42, lines 2-7:
"37. The system according to paragraph 36, wherein said FET labeled oligonucleotide has said quencher domain located at its 3' end.
38. The system according to paragraph 37, wherein said quencher domain comprises a dark quencher."
- on page 43, lines 10-14:

"46. The kit according to paragraph 44, wherein said FET labeled oligonucleotide has said quencher domain located at its 3' end.
47. The kit according to paragraph 46, wherein said quencher domain comprises a dark quencher."

There is thus a direct and unambiguous disclosure for all features of claim 1 in the application as originally filed. The wording of claim 1 corresponds also to the wording used in the application as filed, and does not indicate that the probe may contain two quenchers as suggested by the appellant-opponent. In any case, this cannot amount to a problem under Article 123(2) EPC since similar wording is used in the application as filed.

As to claim 11, this claim refers to a kit comprising said FET-labeled oligonucleotide, and thus the conclusions reached for claim 1 apply *mutatis mutandis* to claim 11.

1.1.2 The subject-matter of **claims 3, 4 and 15**

The subject-matter of granted dependent claims 3, 4 and 15 read:

"3. The FET labeled probe according to claim 1 and claim 2, wherein said nucleic acid intercalator provides increased stability to the hybrid formed from said FET labeled oligonucleotide.

4. The FET labeled probe according to any preceding claim, wherein said nucleic acid intercalator provides exonuclease activity resistance to said FET labeled oligonucleotide.

15. The kit according to claim 13 or claim 14, wherein said minor groove binder provides increased stability to the hybrid formed from said FET labeled oligonucleotide."

The subject-matter of these claims is to be found directly and unambiguously respectively in claims 4, 5 and 17 as originally filed in relation with the claimed FET-labeled probe or a kit comprising it of original claims 1 and 13. The subject-matter of claims 3, 4 and 15 therefore cannot go beyond the application as originally filed and is disclosed directly and unambiguously in the application as filed.

The interpretation of the appellant-opponent that, in view of their dependent claims 3, 4 and 15, independent claims 1 and 11 encompassed also a FET labelled probe having the structural features recited in said claims 1 and 11 but without the properties recited in said dependent claims 3, 4 and 15, is irrelevant and unrelated to the requirements of Article 123(2) EPC; it is indeed sufficient to demonstrate that the subject-matter of said independent claims 1 and 11 does not go beyond the subject-matter of the original application, which is the case (see point 1.1.1 above).

1.1.3 The subject-matter of **claim 10**

Said claim reads:

"10. The FET labeled probe according to claim 9, wherein said FET labeled oligonucleotide is a probe selected from the group consisting of: Taqman probes, scorpion probes, sunrise probes, molecular beacons, conformationally assisted probes, and in situ hybridization probes."

The subject-matter of claim 10 is disclosed directly and unambiguously in original claim 12, which is dependent on claim 1 as originally filed relating to the claimed FET-labeled probe.

1.1.4 Conclusion

All the features or claims objected to are derivable directly and unambiguously from the application as filed, and the main request meets the requirements of Article 123(2) EPC.

1.2 Article 76(1) EPC

1.2.1 Feature **"the nucleic acid intercalator comprises a polycyclic compound"** in claims 1 and 11.

A reference to the cyclic structure of the nucleic acid intercalator is to be found in the description of the parent application WO 03/072051 on page 7, lines 4-5 and on page 15, line 28-33 with following respective wordings:
- "nucleic acid intercalators are aromatic compounds having a flat configuration, and are preferably polycyclic"
- "...nucleic acid intercalators of interest are aromatic compounds having a flat configuration. They may be cyclic, or polycyclic, particularly polycyclic aromatic having at least two rings...".

It is clear from these explanations that the configuration of the intercalator can only be flat, such flat configuration requiring that said intercalator consists of a polycyclic structure. A

structure which "comprises a polycyclic compound" cannot however guarantee such flat configuration.

There is thus no basis in the parent application WO 03/072051 for the feature "a nucleic acid intercalator which comprises a polycyclic compound", as regards the term "comprises" in claims 1 and 11 of the main request.

1.2.2 Feature "**dark quencher**" and "**wherein the dark quencher is located at the 3'end**" in claims 1 and 11.

Claim 10 of the parent application relates to a method involving "a FET labeled oligonucleotide that includes a 3'-5' exonuclease resistant quencher domain comprising a dark quencher". Furthermore it is specified in claim 14, dependent on claim 10, that "said dark quencher is located at the 3'end of said FET labeled oligonucleotide". The same basis can also be found in claims 36-38, 44 and 46-47 of the parent application (see WO 03/072051) referring to a system and a kit comprising said FET-labeled oligonucleotide.

The features "dark quencher" and "wherein the dark quencher is located at the 3' end" are therefore derivable directly and unambiguously from the parent application.

1.2.3 The wording of claim 1

The remaining features of claim 1 not discussed under points 1.2.1 and 1.2.2 above are:
i) "a FET labeled probe comprising a nucleic acid intercalator ...wherein said nucleic acid intercalator is covalently bonded to the 3' end of said FET labeled oligonucleotide ...", and further

ii) "wherein said FET labeled probe is 3'->5' exonuclease resistant."

The parent application WO 03/072051 relates to a FET-labelled oligonucleotide and methods, systems or kit comprising it, said FET-labeled oligonucleotide being in particular "3'->5' exonuclease resistant" (see page 12, lines 2-3). The description mentions further on page 15, lines 26-27 that "in many embodiments the intercalator is covalently bonded to the 3' end".

The remaining features of claim 1 as granted are therefore derivable directly and unambiguously from the parent application.

There is thus a direct and unambiguous disclosure for all features of claim 1 in the parent application. The wording of claim 1 corresponds also to the wording used in the application as filed, and does not indicate that the probe may contain two quenchers as suggested by the appellant-opponent.

1.2.4 The subject-matter of **claims 3, 4 and 15**

The description of the parent application on page 15, lines 17-27 reads:
"covalent binding of nucleic acid intercalators of interest also serve **to increase hybrid stability by providing additional** binding energy.... In addition, nucleic acid intercalators provides **exonuclease activity resistance** when added to the 3' end of said FET-labeled oligonucleotide".

Since claim 1 mentions the covalent bond between the nucleic acid intercalator to the 3' end of the FET-labeled oligonucleotide, said passage of the

description of the parent application is a direct and unambiguous disclosure of the subject-matter of dependent claims 3 and 4.

As to the feature "said minor groove binder provides increased stability to the hybrid formed from said FET labeled oligonucleotide" in claim 15, the description of the parent application refers to a minor groove binder *inter alia* on page 16, lines 30-32, where it is stated that "these atomic interactions stabilize the DNA-minor groove binder structure and, in turn, effectively strengthen the interaction of the two DNA strands of the helix". There is no reference to an increased stability to the hybrid in this passage, and there is thus no basis in the parent application for the feature of claim 15.

As to the appellant-opponent's objections that, in view of their dependent claims 3, 4 and 15, independent claims 1 and 11 encompassed also a FET labelled probe having the structural features recited in said claims 1 and 11 but without the properties recited in said dependent claims 3, 4 and 15, the considerations as set out under Article 123(2) EPC apply also in the context of Article 76(1) EPC (see point 1.1.2, last par.). This objection is thus irrelevant and unrelated to the requirements of Article 76(1) EPC.

1.2.5 The subject-matter of **claim 10**

The subject-matter of this claim is disclosed in claim 12 of the parent application, with the exception of term "in situ hybridization probes", for which there is no basis in the parent application. The subject-matter of this claim thus goes beyond the content of the parent application.

1.2.6 Conclusion

The main request does not meet the requirements of Article 76(1) EPC, as regards some features of claims 1, 10, 11 and 15.

2. Auxiliary request 1

2.1 Amendments (Articles 123(2) and 76(1) EPC)

Auxiliary request 1 differs from the main request by the following amendments:
- the subject-matter of independent claims 1 and 11 specify that the nucleic acid intercalator "**is** a polycyclic compound".
- the deletion of the features "scorpion probes" and "in situ hybridization probes" in dependent claim 10,
- the complete deletion in its entirety of the subject-matter of claim 15 of the main request, and the renumbering of the subsequent claims.

Since these amendments correspond to all features which contravened the requirements of Article 76(1) EPC mentioned above, and since no new subject-matter has been added, auxiliary request 1 meets the requirements of Articles 123(2) and 76(1) EPC.

2.2 Sufficiency of disclosure (Article 100(b) EPC)

The subject-matter of claims 1 and 11 relates to a compound, namely a FET-labeled probe, or a kit comprising it. The structure of the FET probe is disclosed in the description, in particular in paragraphs [0038] to [0049]. The preparation of some specific probes is furthermore disclosed in paragraph

[0106]. In the absence of arguments or evidence, it is not credible that a skilled person would not be able to prepare the probes of claim 1 or the kits comprising it in claim 11.

As to the objection of lack of disclosure in respect of the achievement of certain technical effects, this objection and argumentation are irrelevant in the present case. The claimed subject-matter relates exclusively to a product, and no effect or result-to-be achieved set out in claims 1 or 11. This means that any effect is part of the problem to be solved, and thus of the assessment of inventive step, and appears to be irrelevant to the question of disclosure.

2.3 Clarity (Article 84 EPC)

The appellant-opponent raised an objection of lack of clarity on the grounds that "scorpion probes" or "sunrise probes" as claimed in dependent claim 10 of the main request cannot have a dark quencher on the 3’ terminus, since these probes already contain a PCR primer attached 3' to the quencher. According to the appellant-opponent, the dark quencher cannot be at the 3’ terminus as claimed.

The feature "scorpion probes" has been deleted from the subject-matter of claim 10 of auxiliary request 1, and now only the "sunrise probes" are still claimed. This feature was however already present in claim 10 as granted and therefore is not open to examination for lack of clarity (decision G 3/14).

Moreover, there is no mention in dependent claim 10 of a 3' terminus position of the dark quencher, but rather of a 3' end position. Lastly, no evidence in support of

this objection has been brought by the appellant-opponent about the incompatibility of such probe with the presence of a dark quencher at the 3' end.

2.4 Inventive step

2.4.1 The invention relates to a 3'-> 5' exonuclease resistant FET-labeled oligonucleotide that includes a dark quencher (DQ) and a nucleic acid intercalator (IC) located at the 3' end of the oligonucleotide. This FET-labeled probe can be used in the polymerase chain reaction (PCR), and in particular high fidelity real-time PCR. The claimed structure includes a further fluorophore, which may be positioned anywhere.

2.4.2 In the decision under appeal, D1 was considered as closest prior art. It discloses on page 64 the following FET-labelled probes with the fluorophore FAM at the 5' end and with a dark quencher BHQ1 or DABCYL at the 3' end:
- 5’-FAM-oligonucleotide-BHQ1-3’.
- 5’-FAM-oligonucleotide-DABCYL-3’.
Said structures do not comprise a nucleic acid intercalator covalently bonded to the 3' end. D1 mentions however on page 41 the possibility of including a nucleic acid intercalator, as well as a minor groove binder (MGB).

During appeal proceedings, D24 was also put forward for the first time as an alternative starting point for the assessment of inventive step by the appellant-opponent. This document discloses a FET oligonucleotide probe with 5' fluorophore (FL), a 3' dark quencher (DQ) and a minor groove binder (MGB) attached to the quencher moiety at the 3' end (see page 8, lines 20-30, claims 22, 30 and Figure 1):

5'- FL-oligonucleotide-DG-MGB-3'.
Said structures do not comprise a nucleic acid intercalator covalently bonded to the 3' end.
D24 mentions further that the minor groove binder improves the binding and discrimination characteristics particularly in the TaqMan PCR assay of single nucleotide polymorphism, and that the enhanced ability of MGB-oligonucleotide conjugates to allow discrimination between a perfect hybrid and a hybrid containing a single-base mismatch facilitates the use of hydrolyzable probe assays in the identification of single-nucleotide polymorphisms and the like (see page 40, lines 22-26).

In view of the presentation of these multiple routes by the appellant-opponent, the Board will evaluate inventive step in respect of both documents D1 and D24.

2.4.3 According to the appellant-proprietor, the problem to be solved is the provision of a FET-labeled probe with an improved signal-to-noise ratio and a resulting improved allele discrimination.

2.4.4 As a solution to this alleged problem, claims 1 and 11 of auxiliary request 1 propose a FET probe comprising in particular a nucleic acid intercalator bonded to the 3' end of the FET-labeled oligonucleotide.

2.4.5 It has to be investigated whether there is sufficient evidence supporting the alleged effect over the teaching of documents D1 and D24.

(a) The patent in suit provides in example 6 a comparison of the cross-reactivity or noise in allele discrimination under high fidelity PCR between probes all having a dark quencher (BHQ1)

located at the 3' end, having a fluorophore (FAM or TET) located at the 5' end, and in which furthermore a nucleic acid intercalator (**ACR**) is located at the 5' end, at the 3' end or is absent. Table 6 of Example 6 makes possible the following comparisons:
i) 5'-FAM-oligonucleotide-**ACR**-BHQ1-3' according to the invention compared to 5'-FAM-oligonucleotide-BHQ1-3'
ii) 5'-TET-oligonucleotide-**ACR**-BHQ1-3' according to the invention against 5'-TET-**ACR**-oligonucleotide-BHQ1-3' and 5'-TET-oligonucleotide-BHQ1-3'.

This example shows in comparisons i) and ii) that the presence of the nucleic acid intercalator (ACR) at the 3' end results in reduction of the cross-reactivity or noise from 32% to 2% when the fluorophore is FAM, and from 46% to 4% when the fluorophore is TET, in comparison to a probe which does not contain any intercalator (see Table 6). When the intercalator is located at the 5' end for a probe containing TET as fluorophore, the noise level is 30%.

As shown in Table 7, the percentage of dominant allelle ΔRn to total ΔRn is 98% when the intercalator is present at 3' end, and only 88% or 93% when the intercalator is absent.

(b) Example 6 therefore provides a direct comparison between the FET probes as disclosed in D1 or even probes with an intercalator located at the 5' end with the FET probes as claimed.
It is furthermore clear that the probes according to the invention used in Example 6 fall within the the claim scope, since claim 1 encompasses a FET

probe wherein the intercalator (ACR) is acting as a linker between the BHQ1 and the oligonucleotide.

It is also clear that the claimed FET probe of claim 1 is broader than the exemplified probes of Example 6. However, in the absence of any counter-evidence or reasoned argumentation, the Board considers it credible that substantially all the probes covered by claim 1 have the effect shown in Example 6.
In this regard, the technical explanations given by the appellant-opponent, namely that it was not known whether the effect observed was due to intercalation or to participation of the ACR in Π-stacking, or to a different digestion process, that the design of the experiment was not suitable to show that positioning of ACR at the 3' end was important for the effect, remain speculative in the absence of any concrete experimental supporting evidence showing that they are founded (cf. D52 and Annex B). Annex B can in particular not be taken into consideration, since the FET probes disclosed therein do not comprise any dark quencher and have their intercalator located at the 5' end.

The Board is also not convinced by the appellant-opponent's argument that the data of Example 6 could not support the technical effect of allele discrimination, since they were not provided in the context of a competitive setting. This argument is not supported by any evidence and also lacks credibility, since the skilled person would expect at least a proportionality of the results shown in Example 6 following transposition into a competitive setting.

The alleged technical effect has therefore been shown over the FET probes disclosed in D1.

(c) Example 6 does not however provide a comparison between the FET probes disclosed in D24 and the probes of claim 1. It is thus not possible to establish the existence of an improvement over D24 on the basis of said example 6. Consequently, in the absence of any experimental evidence showing the alleged technical effect over the probes of D24, the technical problem over D24 must be reformulated as the provision of alternative FET-labelled probes. In view of the information found in the examples of the contested patent, the Board is convinced that the problem has been plausibly solved.

2.4.6 It remains to be determined whether the solution was obvious to the person skilled in the art.

(a) Obviousness with regard to D1 as closest prior art

D1 mentions the possibility of including intercalating agents in its FET probes, stating that "the nucleic acid can be modified at the base moiety, sugar moiety, or phosphate backbone with other groups such as radioactive labels, minor groove binders, intercalating agents, donor and/or acceptor moieties and the like" (see page 41, lines 15-20). There is however no disclosure or suggestion in D1 regarding the type of intercalating agents to be used. In particular, there is no teaching to use polycyclic intercalators which are able to form a flat configuration. There is also no information as to their position in the probe. Moreover, the location of the dark quencher could be also at the 5' end (see page 43, lines 5-11).

There is also no disclosure in any of the documents cited by the appellant-opponent, namely D2, D6, D7, D24, D33, D41 and D50 that a FET probe provided with a nucleic acid intercalator located at the 3' end might have a benefit in terms of the signal-to-noise ratio and a resulting improved allele discrimination. None of said documents discloses a FET probe with an intercalator located at the 3' end of an oligonucleotide:

(i) D2 relates to FET binary clamp probes that might comprise a quencher, such as *inter alia* the dark quencher DABCYL, said probes having a different structure than the structure claimed in claim 1 of auxiliary request 1; the arrangement in D2 shows the fluorophore on one oligonucleotide, and the quencher and minor groove binder on another oligonucleotide (see Figure 5; p. 19, l. 10; p. 21, l. 12-14). Intercalators are furthermore mentioned as hybridization stabilization moieties, without any indication about their structure and possible location on said FET probe (p. 12, l. 20).

(ii) D6 relates to arabinofuranosyl-oligonucleotides useful as diagnostic probes, and mentions the possibility of inserting intercalators at an unspecified location in said oligonucleotides to increase the binding affinity of the oligonucleotide for its target sequence and to enhance the stability of the complementary sequence complex (see col. 4, l. 48-68). There is no suggestion in D6 to use such intercalators in FET probes.

(iii) D7 is a review article dealing with conjugates of oligonucleotides useful as linkers, probes, and primer in activities such as sequencing, amplification by PCR, determination of secondary structure engineeering mutations, tailoring RNA with ribonuclease H and assembling DNA constructs (see page 165). D7 mentions i.e. that intercalators are used to strengthen the hybridization of the oligonucleotide with its complement and polylysine used to enhance cellular uptake (see page 166), and that this intercalation can take place at the 3' end (see page 175). D7 provides no teaching in relation to the problem of increasing the signal-to-noise ratio.

(iv) D24 discloses oligonucleotide with a fluorophore, a dark quencher and a minor groove binder. There is no mention or suggestion of using a nucleic acid intercalator in this document.

(v) D33 relates to a method of synthesis of oligonucleotides having tail molecules joined at the 3'terminus, and highlights that an acridine 3' intercalation improved the stability and exonuclease resistance of oligonucleotides (see col. 1 l. 16-41; col. 3, l. 3-9). This document does not refer to FET probes.

(vi) D41 discloses the 3' and/or 5' covalent binding of acridine to an oligonucleotide and the influence of the linker and attachment location on the binding properties of said conjugate with its DNA counterpart (see abstract; Table 2 and page 376, last par. - page 377, first

par.). It shows that when acridine is covalently linked to either the 3', the 5' or both the 3' and 5' ends, the Tm values and thus the corresponding binding properties with the DNA counterpart are not very different (see Table 2 and pages 376-377). This document does not refer to FET probes.

(vii) D50 relates to a specific FET probe comprising cholesterol moieties, which may comprise hybridization enhancers, such as intercalators or minor groove binders, the latter being preferred (see page 26, l. 29 - p. 27, l.2).

The provision of a FET comprising in particular a nucleic acid intercalator bonded to the 3' end of the FET-labeled oligonucleotide is therefore not obvious over D1, alone or in combination with any of the cited documents.

(b) Obviousness with regard to D24 as closest prior art

The teaching of D24 is restricted to the use of a minor groove binder. There is no disclosure or suggestion in D24 either to replace the disclosed minor groove binder with a nucleic acid intercalator at the 3' end location, or to insert such intercalator at said 3' end location. Moreover, said minor groove binder is used to improve the binding and also the discrimination characteristics of the disclosed conjugate. The improvement of the discrimination characteristics by nucleic acid intercalators is not disclosed or suggested in any of the cited documents D24 or D2, D6, D7, D33, D41 or D50 (see point (a) above).

The skilled person would therefore not see any hint in the teaching of D24 to replace the minor groove binder with an intercalating agent.

The Board could in particular not follow the argumentation of the appellant-opponent that a minor groove binder and an intercalator are interchangeable. It is in fact common general knowledge that the two categories of products have different structures, functions and mechanisms of action:
- DNA intercalators are known to be molecules capable of fitting between nucleic acid base pairs, in view of their ability to be inserted between DNA base pairs, thereby interrupting DNA functions and inducing structural distortions. The intercalators are typically chemotherapeutic agents (see for instance D45).
- Minor groove binders are non-intercalating molecules capable of binding isohelically into the minor groove of double-stranded DNA by fitting into said minor groove, thereby causing only little distortion of the DNA backbone; minor groove binders are known to increase the sequence specificity (see the description of the contested patent, par. [0059]-[0062]; see also D21, claim 1 and D23). Minor groove binders have a crescent-shaped three-dimensional structure, as illustrated by the compounds identified in the patent in suit (see par. [0062]).

Thus, even if said minor groove binders and intercalators might share some properties, such as stabilisation of the hybridization complex (see D2, page 12, lines 19-21), the mechanisms of action involved and their use are different. They therefore represent two distinct categories of molecules. The skilled person would not replace a compound from one

category with a compound belonging to the other category.

As mentioned under point (a) above, there is additionally no disclosure or suggestion in any cited prior art document to prepare a FET- labeled probe having a nucleic acid intercalator located at the 3' end of the oligonucleotide.

The provision of a FET comprising in particular a nucleic acid intercalator bonded to the 3' end of the FET-labeled oligonucleotide is therefore not obvious over D24, alone or in combination with any of the cited documents.

2.4.7 Consequently, auxiliary request 1 meets 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 maintain the patent on the basis of auxiliary request 1 filed with letter of 16 January 2017 and a description to be adapted thereto.

The Registrar: The Chairman:

S. Fabiani A. Usuelli

Decision electronically authenticated