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Case Number: T 0518/22 - 3.3.08

Application Number: 10805180.6

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Language of the proceedings: EN

Title of invention:
Thermostable Type-A DNA polymerase mutants with increased polymerization rate and resistance to inhibitors

Patent Proprietor:
Agilent Technologies, Inc.

Opponent:
Roche Diagnostics GmbH

Headword:
Type-A DNA polymerase mutants/AGILENT

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 87, 123(2), 123(3)
RPBA 2020 Art. 12(4), 13(2)

Keyword:

Main request and auxiliary request 2 - added subject-matter - (yes)
Undisclosed disclaimer - not allowable
Auxiliary request 1 - novelty - (no)
Auxiliary request 3 - requirements of the EPC met - (yes)

Decisions cited:

G 0001/03, G 0003/14, G 0001/15, G 0001/16, G 0002/21, G 0001/22, G 0002/22, T 0032/84, T 0292/85, T 2790/17, T 1975/19, T 0025/20, T 0559/20, T 2164/21, T 0521/18

Catchword:

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Case Number: T 0518/22 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 30 October 2024

Appellant I: (Patent Proprietor)	Agilent Technologies, Inc. 5301 Stevens Creek Boulevard Santa Clara, CA 95051 (US)
Representative:	Grammel, Markus und Seisenberger Georg Grünecker Patent- und Rechtsanwälte PartG mbB Leopoldstraße 4 80802 München (DE)
Appellant II: (Opponent)	Roche Diagnostics GmbH Sandhoferstr. 116 68305 Mannheim (DE)
Representative:	Teschemacher, Andrea Simmons & Simmons LLP Lehel Carré Thierschplatz 6 80538 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 20 December 2021 concerning maintenance of the European Patent No. 2524036 in amended form**

Composition of the Board:

Chair	T. Sommerfeld
Members:	M. Montrone
	A. Bacchin

Summary of Facts and Submissions

I. Appeals were lodged by the patent proprietor and the opponent ("appellants I and II", respectively) against the interlocutory decision of the opposition division concerning maintenance of the European patent No. 2 524 036 in amended form. This patent is based on European patent application No. 10805180.6 which has been filed as International patent application published as WO 2011/014885 (the "patent application").

II. The opposition proceedings were based on the grounds for opposition in Article 100(a) EPC (in conjunction with Articles 54 and 56 EPC), 100(b) and 100(c) EPC.

III. The opposition division held in the decision under appeal that claim 1 as granted (main request) comprised added subject-matter and that claim 1 of auxiliary request 1 lacked novelty over document D2. Auxiliary request 2 was held to comply with the requirements of the EPC.

IV. In their statement of grounds of appeal (hereinafter "SGA"), appellant I submitted a main request and auxiliary requests 1 to 29. The claim set of the main request was not identical to that of the claims as granted (the feature "*wherein the substitution at residue 507 is a replacement of glutamic acid (E) in wild-type Taq DNA polymerase, or the corresponding residue in the other thermostable Type-A DNA polymerase of any one of SEQ ID NOS: 1-10 to lysine (K)*" was missing from claim 9 of the main request contrary to claim 9 as granted), while auxiliary requests 1 to 29 were identical to those already submitted during the

opposition proceedings, except for being in part re-numbered.

V. In their SGA, appellant II provided arguments under added subject-matter, insufficiency of disclosure, lack of priority, novelty and inventive step against the claims as granted and auxiliary requests 1 and 2.

VI. The parties provided in further submissions replies to each others SGAs.

VII. In a further submission appellant I re-filed claims 1 to 14 as granted as main request.

VIII. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's preliminary opinion.

IX. In reply, appellant I submitted further arguments.

X. Oral proceedings were held as videoconference in the presence of all parties. During the oral proceedings, appellant I renumbered auxiliary request 5 filed with the SGA as auxiliary request 3 while the remaining auxiliary requests were renumbered accordingly.

XI. Claims 1, 7 to 9 and 14 of the main request (claims as granted) read:

"1. A mutant thermostable Type-A DNA polymerase consisting of or comprising:
a first mutation at residue 507 of wild-type Taq DNA polymerase which has the sequence of SEQ ID NO: 4 or at a residue corresponding to residue 507 of wild-type Taq DNA polymerase in another thermostable Type-A DNA polymerase of any one of SEQ ID NOS: 1-10; and

at least one additional mutation at a residue selected from 59, 155, 245, 375, 508, 734, and 749 of wild-type Taq DNA polymerase, or at a corresponding residue in another thermostable Type-A DNA polymerase of any one of SEQ ID NOS: 1-10,
wherein the combination of mutations provides a mutant polymerase that possesses a faster polymerization rate and a higher resistance to polymerization activity inhibitors than the wild-type DNA polymerase from which it is derived, and
wherein the substitution at residue 507 is a replacement of glutamic acid (E) in wild-type Taq DNA polymerase or the corresponding residue of the other thermostable Type-A-DNA polymerase of any one of SEQ ID NOS: 1-10 to lysine (K)."

"7. A mutant Taq DNA polymerase consisting of or comprising the sequence of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, or SEQ ID NO:38, wherein the polymerase possesses a faster polymerization activity than wild-type Taq polymerase and is resistant to inhibitors of wild-type Taq polymerase.

8. A kit for amplification of a target nucleic acid, said kit comprising the mutant DNA polymerase of any one of claims 1-7 and packaging materials therefor.

9. A method of polymerization of a target nucleic acid from a primer that specifically binds to the target nucleic acid, said method comprising:
combining the primer with the target nucleic acid and a mutant thermostable Type-A DNA polymerase,
wherein the mutant thermostable Type-A DNA polymerase consists of or comprises:

a first mutation at residue 507 of wild-type Taq DNA polymerase which has the sequence of SEQ ID NO: 4 or at a residue corresponding to residue 507 of wild-type Taq DNA polymerase in another thermostable Type-A DNA polymerase of any one of SEQ ID NOS: 1-10; and
wherein the substitution at residue 507 is a replacement of glutamic acid (E) in wild-type Taq DNA polymerase or the corresponding residue of the other thermostable Type-A-DNA polymerase of any one of SEQ ID NOS: 1-10 to lysine (K); and
at least one additional mutation at a residue selected from 59, 155, 245, 375, 508, 734, and 749 of wild-type Taq DNA polymerase, or at a corresponding residue in another thermostable Type-A DNA polymerase of any one of SEQ ID NOS: 1-10,
wherein the combination of mutations provides a mutant polymerase that possesses a faster polymerization rate and a higher resistance to polymerization activity inhibitors than the wild-type DNA polymerase from which it is derived, and
providing conditions under which the polymerase extends the primer using the sequence of the target as a template for incorporation of nucleotides."

"14. An isolated DNA molecule comprising a nucleotide sequence that encodes the mutant polymerase of any one of claims 1-7".

The remaining claims 2 to 6 and 10 to 13 are dependent on one or more of the independent claims.

XII. Claims 1 and 9 of auxiliary request 1 differ from claims 1 and 9 of the main request in that the other thermostable Type-A DNA polymerases have been limited to "*SEQ ID NOS: 4-8*".

XIII. Claims 1 and 9 of auxiliary request 2 differ from claims 1 and 9 of auxiliary request 1 in that the disclaimer "*provided that the mutant thermostable Type-A DNA polymerase is not the Type-A DNA polymerase shown in SEQ ID NO: 9 of WO 2010/062777*" has been added.

XIV. Claims 1 and 9 of auxiliary request 3 differ from claims 1 and 9 of the main request in that the embodiments in relation to "*another thermostable Type-A DNA polymerase of any one of SEQ ID NOS: 1-10*" have been deleted and in that the disclaimer "*provided that the mutant thermostable Type-A DNA polymerase is not the Type-A DNA polymerase shown in SEQ ID NO: 9 of WO 2010/062777*" has been added. Furthermore, claim 9 of auxiliary request 3 differs from the respective claim as granted in that the alternative "*or comprises*" has been deleted from the feature "*wherein the mutant thermostable Type-A DNA polymerase consists of or comprises*".

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

P: US 61/230,275 (priority document of the patent)

P1: US 61/110,877 (priority document of D2)

D1: US 2003/0186238 A1

D2: WO 2010/062777

D3: WO 01/90337

D4: Kermekchiev M. *et. al.*, Nucleic Acids Research, 2009, Vol. 37(5), e40, 1-14

D7: Arezi B. *et. al.*, Frontiers in Microbiology, 2014, Vol. 5, Article 408: 1-10.

XVI. Appellant I's submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request

Claim construction

The polymerisation rate (i.e. speed) of the claimed polymerase mutants was a kinetic parameter that was independent from the mutants' enzymatic activity. Both parameters were separately determined (number of nucleotides incorporated per unit time versus the number of units per milligram of enzyme converting a substrate, respectively).

An inhibitor affected the activity of an enzyme by interfering with the enzyme's affinity for the substrate. If the enzyme's activity was blocked, measurements of its speed were no longer possible. This was another indication that both enzymatic parameters were independent of each other, since the enzyme activity was dependent on the enzyme's substrate affinity contrary to the polymerisation speed.

Added subject-matter

The generic 507K mutation in polymerases encoded by SEQ ID NOs: 1 to 3, 9 and 10 of claim 1 had a basis in paragraphs [012], [016], [034], [049], [050] and [055]. Paragraph [050] mentioned in general that non-Taq polymerases had one or more mutations at residues corresponding to Taq. Moreover, paragraph [055] disclosed that the E507K mutation in Taq was

responsible for the enhanced speed of the mutated enzyme. The presence of K was thus mandatory at position 507 in Taq for increasing its speed and K was mentioned as sole amino acid at this position. This indicated to the skilled person taking the application as filed as a whole into account that K had to be present in all other claimed non-Taq polymerases at the position corresponding to Taq 507 too irrespective of the wild type ("wt") amino acid at this position.

Auxiliary request 1

Priority of document D2

Document D2 was not entitled to claim priority from P1 since the applicants of P1 and those of the subsequent patent application were not the same.

Novelty

Document D2 disclosed that the A3 Taq mutant had mutations falling within claim 1 (E507K and L245M) but also further mutations not mentioned in claim 1. It belonged to the common general knowledge that these additional mutations in A3 could have negatively affected the mutant's functional properties. The A3 Taq mutant thus did not implicitly or inherently disclose the functional properties of the claimed polymerase mutants based on its sequence alone. Since Tables 12 and 15 of D2 did not determine the polymerisation rate (i.e. the speed) of A3 and wt Taq but only their produced amounts of PCR fragments (i.e. their enzyme activities), these Tables allowed no direct and unambiguous conclusions about A3's speed compared to wt Taq. The absence of a PCR product when wt Taq was used under the conditions tested could be caused by several

reasons: a low speed of wt Taq, a low activity, or a too low detection sensitivity of the assay used. Document D2 further reported that the increased binding affinity of the Taq mutants for their templates compared to wt Taq was decisive for their increased inhibitor resistance (paragraph [0098]). An inhibitor-induced lower binding affinity affected Taq's activity but not necessarily its speed. Moreover, the skilled person knew that the polymerisation rate mentioned in claim 1 and Taq's enzyme activity were independent enzymatic properties. This was also confirmed by Figure 1A and Table 2 of document D7 which disclosed that under slow and fast cycling conditions, Taq mutants and wt Taq produced comparable or different DNA amounts.

Auxiliary request 2

Admittance of a new line of argument under partial priority

Since the board in its preliminary opinion was of the view that the embodiment of claim 1 in reference to SEQ ID NO: 4 was entitled to claim priority from P, claim 1 was at least entitled to a partial priority. Appellant II's new line of arguments in relation thereto raised for the first time at the oral proceedings was too late and hence inadmissible, including any related arguments under lack of novelty over document D2 of claim 1's "comprising" embodiment concerning SEQ ID NOs: 5 to 8.

Added subject-matter

The embodiment of claim 1 in reference to SEQ ID NO: 4 was entitled to claim priority from P. Also claim 1's embodiments in reference to SEQ ID NOs: 5 to 8 were entitled to claim priority from P since they were

unambiguously derivable from claims 1, 2, 10 and paragraphs [015], [024], [062] and Figure 1 of P.

The disclaimer in claim 1 excluded the disclosure of document D2 which was prior art under Article 54(3) EPC for the claimed embodiments in reference to SEQ ID NO: 4 that were entitled to claim priority from P. Document D2 solely disclosed Taq Type-A polymerase mutants. Since the polymerases in reference to SEQ ID NOs: 5 to 8 in claim 1 were non-Taq polymerases, document D2 did not anticipate mutants derived therefrom. Thus irrespective of whether document D2 was prior art under Article 54(2) EPC for the embodiments in claim 1 in reference to SEQ ID NOs: 5 to 8, the disclaimer in claim 1 comprised no added subject-matter.

Auxiliary request 3

Admittance of auxiliary request 3 into the appeal proceedings

Auxiliary request 3 was already submitted during the opposition proceedings and re-submitted with the SGA. The amendment simply consisted in narrowing down the subject-matter of auxiliary request 2 and the purpose was straightforward, namely to overcome the lack of novelty and the finding of non-allowability of the disclaimer. Moreover, reasons for admitting auxiliary request 3 were submitted with the SGA and in the letter submitted in response to appellant II's reply to appellant I's appeal. This set of claims was thus admissible.

Sufficiency of disclosure

T 2164/21 was irrelevant for the present case since in that case evidence was available that antibodies falling within the claimed scope did not have the claimed functional properties. In the present case, however, appellant II had failed to provide such evidence. Further the application as filed disclosed that the E507K mutation caused Taq's increased polymerisation rate and that Taq's increased inhibitor resistance correlated with the other mutations mentioned in claim 1. The application as filed disclosed thus a structure-function relationship between the mutations and functional properties of the mutated polymerases mentioned in claim 1. This was confirmed by post-published evidence (document D7). The mere breadth of claim 1 was no argument under insufficiency. Since moreover the application as filed disclosed the structural data of the polymerases claimed and all assays required for assessing the claimed functional properties, the skilled person would have obtained substantially all polymerases falling within claim 1 without undue burden.

Novelty

Appellant II's new line of argument under lack of novelty against the subject-matter of claim 7 was raised for the first time with their SGA. Since the subject-matter of claim 7 had not changed since the onset of the opposition proceedings, this line of argument should have been submitted earlier and was hence inadmissible.

Document D1 disclosed various polymerases that all contained the D758N mutation that destroyed the enzymes' polymerisation activity. Already for this

reason, the polymerase mutants of document D1 did not anticipate the claimed polymerase mutants.

The KT 10 polymerase mutant in document D4 was mutated at position 708, i.e. at a completely different site from Taq's residue 507, the mutation required in the claim.

Inventive step

Appellant II submitted a line of argument under lack of inventive step starting from document D3 as closest prior art only, although the opposition division based its reasoning in favour of an inventive step starting from document D4 as closest prior art. Nor was appellant II's line of argument under lack of inventive step based on document D3 complete. This line of argument under inventive step was thus inadmissible.

The claimed polymerase mutants differed from those of document D3 not only structurally (i.e. by the positions indicated in claim 1, except for E507K) but also by their functional properties. Since moreover document D3 was silent on the functional properties of the polymerase mutants mentioned in claim 1, the claimed mutants were inventive.

XVII. Appellant II's submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request

Claim construction

Claim 1 was silent on the conditions under which the polymerisation rate (speed) of the polymerase mutants

were determined. A link existed between the activity of a polymerase and its speed, i.e. an active enzyme was necessarily faster (had an increased polymerisation rate) than an inactive enzyme. Further, since an inhibitor blocked in general an enzyme's activity, the inhibitor indirectly lowered the polymerase's polymerisation rate too.

Added subject-matter

Since paragraphs [012] and [049] of the application as filed did not mention the claimed polymerases, these paragraphs provided no link between the polymerases of SEQ ID NOs: 1 to 10 and an amino acid substitution at position 507 of Taq to K, let alone at a corresponding residue in the other polymerases. Nor was the selection of K at position 507 in Taq in these paragraphs indicated as being preferred.
Paragraph [016] disclosed general statements only that lacked any information about the specific mutation to be made.
Nor was the combination of SEQ ID NOs: 1 to 10 and a mutation at position 507 unambiguously derivable from paragraph [034] of the application as filed.
Paragraph [050] of the application as filed mentioned non-Taq polymerase mutants, while the E507K mutation in paragraph [055] was solely disclosed for Taq (SEQ ID NO: 4). Paragraph [050] disclosed thus no link to the non-Taq polymerases indicated in claim 1, let alone for an E at position 507, or for any amino acid ("X") at position 507. Paragraph [055] did also not disclose non-Taq polymerases with a K at position 507, let alone that any amino acid could be present at position 507 in these polymerases or be mutated except for E.
Although wt polymerases encoded by SEQ ID NOs: 5 to 8 had an E at position 507, a link for mutating E to K at

position 507 in these polymerases was not derivable from any of the paragraphs of the application as filed.

Auxiliary request 1

Novelty

The subject-matter of claim 1 lacked novelty over document D2. Tables 12 and 15 of document D2 disclosed the A3 Taq mutant and its ability to produce a DNA fragment in the presence of various inhibitors (metal ions and phenol), contrary to wt Taq. While document D2 indeed did not determine A3's polymerisation rate, since wt Taq was unable to synthesise any DNA product under the conditions tested, the polymerisation rate of the A3 mutant was necessarily higher compared to Taq.

Auxiliary request 2

Admittance of a new line of argument under partial priority

Appellant I's arguments in relation to partial priority of Type-A DNA polymerase referring to SEQ ID NO:4 were submitted in response to the board's preliminary opinion only. The specific counter arguments submitted during oral proceedings in response thereto could thus not have been submitted earlier and should be admitted into the proceedings.

Priority

The subject-matter of claim 1 encompassed several alternative embodiments. The mutant polymerases in relation to SEQ ID NO: 4 were entitled to claim a partial priority from P. However the mutant polymerases

in relation to SEQ ID NOs: 5 to 8 were not entitled to claim priority from P since these embodiments were not directly and unambiguously derivable therefrom. Document D2 was thus prior art under Article 54(2) EPC for these embodiments of claim 1.

Added subject-matter

Since document D2 was prior art under Article 54(2) EPC for the embodiments of claim 1 in relation to the mutant polymerases with reference to SEQ ID NOs: 5 to 8, such a disclosure in D2 could not be disclaimed from claim 1 without adding subject-matter (G 1/03).

Auxiliary request 3

Admittance of auxiliary request 3 into the appeal proceedings

Appellant I's SGA contained only general statements why auxiliary request 3 was filed. Reasons why the amendments were introduced and why the amended claims complied with the requirements of the EPC were however lacking. The present situation was thus similar to the situation underlying case T 559/20, where requests that had been submitted already in opposition were not admitted in appeal because of lack of substantiation. Appellant I had not presented their complete case in appeal, contrary to the requirements of Article 12(3) and (5) RPBA, and therefore auxiliary request 3 should not be admitted.

Sufficiency of disclosure

Most of the Taq mutants containing the E507K mutation and at least one additional undefined mutation

encompassed by the "consisting" and "comprising" embodiments of claim 1 did not show an increase in their polymerisation rate and inhibitor resistance compared to wt Taq. This was so because based on the common general knowledge, substitutions in polymerases at the claimed positions by an amino acid having a different property than the wt amino acid (e.g. size or charge) negatively affected the enzyme's function. In the absence of any information in the application as filed about amino acids being suitable for such replacements, the skilled person had no guidance for finding alternatives to the three exemplified Taq mutants "*2C2*", "*Taq42*" and "*3B*".
Decision G 2/21 (Reasons 74 and 77) set out that for a second medical use claim complying with the requirements of Article 83 EPC it had at least to be credible that the medical agent was suitable for the claimed therapeutic application based on the data in the application as filed. This standard applied to all functional features mentioned in a claim, not only for second medical use claims see, for example, decision T 2790/17 (Reasons 2.3 and 2.8.5.). Therefore substantiated doubts existed about the potential mode of action of the individual mutation on the claimed functional properties (T 25/20). The application as filed provided only limited experimental data with one 3-fold mutant and two 7-fold mutants which all contained largely overlapping mutations. The data obtained from these mutants were thus insufficient for establishing a credible technical concept applicable across the whole scope of claim 1. For example, the application as filed provided no proof that the combination of the 507K mutation in Taq with a single mutation at one of the other positions indicated in claim 1 resulted in a mutant polymerase showing the claimed properties. According to established case law

these effects had to be achieved without undue burden across the whole scope claimed (T 2164/21).
Due to the high number of mutants falling within the "comprising" embodiment of claim 1, the skilled person was unable to ascertain whether he/she was working within the limits of the claim which resulted in undue burden too.

Novelty

Document D1 disclosed 19 polymerase mutants which all contained the E507K and at least one mutation at positions V155I and E734N. The patent disclosed in paragraph [0045] and Figure 6B that the presence of the E507K mutation in Taq alone was sufficient for causing the functional properties indicated in claim 1. These properties were thus inherent in the E507K mutation. Since document D1 disclosed various polymerase mutants containing the E507K mutation, these mutants necessarily had the claimed functional properties too.

Document D4 disclosed on page 3 a Taq polymerase with a E708K mutation ("*KT 10*") that had the same functional properties as the claimed mutants (Figure 1). Page 4 of document D4 disclosed that KT 10 comprised further mutations at positions 626 and 707.
Although these positions were different from the ones indicated in claim 1, the sequences encompassed by the "comprising" embodiment were not distinguished therefrom. Due to the "comprising", the claimed polymerase sequences encompassed by this embodiment lacked a structural framework (i.e. a reference) so that any of the positions indicated in claim 1 was arbitrary.

Inventive step

Either of documents D2 and D3 represented the closest prior art.
Document D3 disclosed mutant Taq polymerases (abstract, page 127, item 2, page 129, item 3) that contained *inter alia* the mutation E507K and other mutations (page 135, second paragraph, page 137, second and third paragraph). The patent in suit (paragraph [0045] and Figure 6B) disclosed that the mutation E507K alone showed the claimed functional properties.
The claimed mutated Taq polymerases differed from that of document D3 in having at least one further mutation at the positions indicated in claim 1. Since the mutation E507K in Taq alone caused the claimed functional properties, no technical effect was associated with the presence of further mutations.
The objective technical problem was the provision of an alternative mutant Taq polymerase. The solution to this problem was however not based on an inventive step since the provision of alternative Taq mutants belonged to the skilled person's common general knowledge.

XVIII. Appellant I requests:

- that the decision under appeal be set aside and that the patent be maintained as granted (main request), or alternatively that the patent be maintained on the basis of auxiliary request 1, or alternatively, that the opponent's appeal be dismissed (auxiliary request 2) or further alternatively that the patent be maintained on the basis of auxiliary request 3, submitted as auxiliary request 5 with the SGA, or of auxiliary requests 4 and 5, submitted as auxiliary requests 3 and 4, respectively, with the SGA, or of auxiliary requests 6 to 29 submitted with the SGA,

- that new lines of arguments under lack of novelty against the subject-matter of claims 1 and 7 of auxiliary request 2 not be admitted in the proceedings,
- that a line of argument under lack of inventive step using document D3 as closest prior art not be admitted.

XIX. Appellant II requests:
- that the decision under appeal be set aside and that the patent be revoked and
- that auxiliary requests 3 to 29 not be admitted into the proceedings.

Reasons for the Decision

Main request (claims as granted)

Claim construction - claim 1

1. Claim 1 is directed to a "*mutant thermostable Type-A DNA polymerase*".

1.1 This mutated DNA polymerase is structurally defined in that it either consists of or comprises:

- a first mutation: substitution of glutamic acid ("E") at residue 507 of wild-type ("wt") Taq polymerase (SEQ ID NO: 4), **or** at the corresponding residue in polymerases encoded by SEQ ID NOs: 1 to 10 to lysine ("K") **and**
- at least one additional mutation: selected from a residue at positions 59, 155, 245, 375, 508, 734, and 749 of wt Taq polymerase **or** the corresponding

residues in polymerases encoded by SEQ ID NOs: 1 to 10.

1.2 In the following the substitution of E to K at position 507 will be referred to as E507K, or if the wt amino acid residue at position 507 is unknown as X507K.

1.3 The claimed mutant polymerase is further functionally defined by a result to be achieved in that "*the combination of mutations provides a mutant polymerase that possesses a faster polymerization rate and a higher resistance to polymerization activity inhibitors than the wild-type DNA polymerase from which it is derived*".

1.4 Claim 1 refers to mutations by their position within the sequences of SEQ ID NOs: 1 to 10 only, except for E507K (SEQ ID NO: 4) or X507K (SEQ ID NOs: 1 to 3 and 5 to 10) as the first mutation. Claim 1 neither structurally specifies the remaining sequence (for example, by their degree of identity towards their respective wt sequences), nor the at least one additional mutation, except for its respective position within SEQ ID NOs: 1 to 10. Thus, the at least one additional mutation in the wt DNA polymerases defined by SEQ ID NOs: 1 to 10 includes any mutation at the respective positions indicated in claim 1, including substitutions and deletions.

1.5 Claim 1 refers to a "*mutant thermostable Type-A DNA polymerase consisting of or comprising*" the structural and functional requirements as defined in claim 1. According to ordinary claim interpretation, the "*comprising*" embodiment of claim 1 allows for the presence of further mutations (not defined by number,

position or type) in the wt sequences encoded by SEQ ID NOs: 1 to 10 from which the mutants are derived.

1.6 In contrast thereto, the "*consisting*" embodiment of claim 1 limits the claimed mutant DNA polymerases to the specified positions at which the mutations occur, i.e. excludes the presence of further mutations. However, the type of mutation at the respective positions indicated in claim 1 is not defined, except for a K at position 507 (507K). Irrespective of whether or not claim 1 relates to the "*comprising*" or "*consisting*" embodiment, all mutants are further defined by the functional properties set out in claim 1 (point 1.3 above).

2. Claim 1 does not define the functional properties of the mutated DNA polymerases, except that these mutants must possess a "*faster polymerization rate*" and a "*higher resistance*" towards "*polymerisation activity inhibitors*" compared to their respective wt polymerase. Since the terms "*faster*" and "*higher*" are relative and claim 1 is silent on the conditions under which these properties are determined, all thermostable Type-A DNA polymerase mutants possessing the structural characteristics set out above and showing a faster polymerisation rate and increased resistance against a polymerisation activity inhibitor under at least one condition when compared to their respective wt polymerases fall within the scope of claim 1, irrespective of how high that increase is.

2.1 The term "*polymerization rate*" normally defines the number of nucleotides incorporated by a polymerase per unit time (under specified reaction conditions, patent, paragraph [0005]).

2.2 The "*polymerization activity*" of a DNA polymerase directly affects the enzyme's "*polymerization rate*" (patent, paragraph [0034]), i.e. both properties are functionally linked.

3. It was disputed between the parties whether all mutants showing a "*higher resistance*" towards polymerisation inhibitors possessed at the same time a "*faster polymerization rate*" when compared to the respective wt DNA polymerase. In other words, whether a faster polymerisation rate of the claimed mutants was implicit in a higher resistance of the polymerase mutant towards polymerisation inhibitors. Appellant I argued that this was not the case since the properties of a DNA polymerase in relation to its polymerisation rate (i.e. speed) and its production rate (i.e. activity) were separately determined by the skilled person and hence independent properties of the enzyme. Moreover the production rate was largely influenced by the enzyme's binding affinity for the template, contrary to the enzyme's polymerisation rate.

3.1 As set out above, claim 1 does not specify the conditions under which the polymerisation rate of the Type-A DNA polymerase mutants is determined. Nor the conditions for determining their polymerisation activity in the presence of an inhibitor, as long as both properties are increased for the claimed mutants vis-a-vis their respective wt polymerases under at least one experimental condition. Claim 1 relates thus to relative and not absolute functional properties of the claimed mutant polymerases.

3.2 Moreover, as likewise indicated above, the polymerisation rate (speed) of the claimed polymerases is functionally linked to the enzymes' polymerisation

activity (production rate). Consequently any DNA polymerase generating a DNA product under certain conditions does this at a certain "speed", i.e. with a certain polymerisation rate since otherwise no product would be generated. If no product is generated under these conditions, the polymerase shows either no polymerisation rate, or a slow rate so that the time given for generating the product was too short. This applies irrespective of the underlying mechanism causing this effect, for example, the binding affinity, the enzymatic activity, or both.

3.3 Owing to the considerations above, the board is not convinced by appellant I's arguments and concludes that because claim 1 defines relative and not absolute functional properties, an increased resistance towards an inhibitor of a claimed mutant DNA polymerase implies necessarily a faster polymerisation rate of this mutant too when compared to the respective wt polymerase under identical test conditions.

Added subject-matter - claim 1

4. Reference in the following to the application as filed is to the patent application (WO 2011/014885).

5. The board agrees with the opposition division's finding (decision under appeal, point 21.11) that claim 1 as granted comprises added subject-matter as regards the presence of SEQ ID NOs: 1 to 3, 9 and 10.

5.1 In particular, a direct and unambiguous basis is missing in the application as filed for substituting any amino acid at the corresponding position 507 of Taq to K in DNA polymerases encoded by SEQ ID NOs: 1 to 10.

5.2 Appellant I in essence argued that the skilled person applying a mind willing to understand and no formalism when reading the application as filed immediately understood that, for wt Type-A DNA polymerases not having an E at a position corresponding to 507 in wt Taq but a glutamine ("Q": SEQ ID NOs: 1 to 3) or threonine ("T": SEQ ID NOs: 9 and 10), the mutation disclosed must be **Q**507**K** or **T**507**K**. This was so because the application as filed (paragraph [055]) disclosed that the 507K mutation was responsible for an enhanced polymerisation speed of the mutated polymerases, while the second mutation indicated in claim 1 increased the polymerases' resistance towards inhibitors. This teaching was generic and applied to all polymerases encompassed by claim 1 as supported by the statement in paragraph [050] of the application as filed reading: "*Where the mutant DNA polymerase is not derived from Taq DNA polymerase, the mutant polymerase can have one or more mutations at residues corresponding to the residues identified herein with specific reference to Taq polymerase*".

6. This is not convincing. The application as filed (paragraphs [012], [049], [054], [055], [071], [073], and claims 2 to 10 as filed) when disclosing mutations at position 507 of Taq or at the corresponding position in Type-A DNA polymerases other than Taq consistently mentions "*E*" as wt amino acid. This does not change in view of paragraph [055] of the application as filed which discloses solely a Taq mutant and "*E507*" and "*E507K*" without providing a link to any of the non-Taq polymerases encompassed by claim 1. This is supported by paragraph [049] of the application as filed which, although referring to non-Taq polymerases, solely mentions "*E507Q or E507K*" (emphasis added), i.e. the wt E amino acid at position 507. Since moreover the

statement in paragraph [050] of the application as filed (point 5.2 above) is silent on positions or mutations, this paragraph cannot provide any evidence to the contrary. Nor does this change in view of the claim construction set out above.

7. Consequently a substitution of any wt amino acid in SEQ ID NOs: 1 to 3 and 5 to 10 by K at a position corresponding to 507 of wt Taq as defined in claim 1 is not directly and unambiguously derivable from the application as filed. The main request therefore adds subject-matter and Article 100(c) EPC prejudices the maintenance of the patent as granted.

Auxiliary request 1

8. Claim 1 of auxiliary request 1 differs from claim 1 as granted in that the claimed mutant DNA polymerases have been limited with reference to SEQ ID NOs: 4 to 8.

Novelty - claim 1

9. Document D2, published on 3 June 2010, so before the patent's filing date (2 August 2010), is an Euro-PCT application filed on 3 November 2009 and therefore after the filing date of the present patent's priority document P (31 July 2009) but claiming an earlier priority date (P1, filed 3 November 2008). If document D2's priority claim based on P1 is valid, D2 is prior art pursuant to at least Article 54(3) EPC.

Priority entitlement of document D2

10. Appellant I objected in writing to document D2's formal entitlement to priority because the applicants of P1 and those of the international patent application D2

were not the same. Since at the oral proceedings no further arguments were submitted by appellant I, the board maintains its preliminary opinion set out in the communication under Article 15(1) RPBA.

10.1 Decisions G 1/22 and G 2/22 (OJ 2024, 50) established that a presumption exists that a claim to priority is valid by way of an implicit agreement on the transfer of the right to claim priority in the absence of evidence that such an agreement (implicit or explicit) did not exist (G 1/22, Reasons 99, 105 to 107, 122 and 125 to 127). This presumption applies to any case where the subsequent applicant is not identical with the priority applicant (G 1/22, Reasons 107 and 134). On account of this general teaching in G 1/22, the board understands that the presumption of validity of the priority claim applies also to patent applications cited as prior art as in the present case (see also T 521/18, Reasons 2.1 to 2.5).

10.2 This presumption can be rebutted to take into account "*rare exceptional cases*" where the subsequent applicant cannot justifiably rely on the priority (G 1/22, Reasons 108 and 131). This, however, involves the reversal of the burden of proof, i.e. the party challenging the subsequent applicant’s priority entitlement (here appellant I) has to prove that this entitlement is missing. Merely raising speculative doubts is not sufficient, instead evidence is required that specific facts support serious doubts about the subsequent applicant's entitlement to priority (G 1/22, Reasons 110, 113 and 126).

10.3 In the absence of evidence suitable to establish that the alleged real priority right holder did not allow the subsequent applicant to rely on the priority (see

also T 1975/19, Reasons 1.1.2), appellant I's objection against document D2's formal entitlement to priority from P1 is not sufficient to rebut the presumption of validity, which always exists on the date on which priority is claimed (G 1/22, Reasons 109).

10.4 Hence, document D2 validly claims priority from P1.

11. It was a matter of dispute between the parties whether the "*A3*" Taq mutant disclosed in document D2 (e.g. Table 3 on page 29) directly and unambiguously anticipated the subject-matter of claim 1.

12. In this respect it was undisputed that Table 3 of document D2 disclosed Taq mutants which were assessed for their resistance to high salt conditions (Table 3, title) and in that the A3 mutant comprised eight point mutations including "*E507K*" and "*L245M*", i.e. the mandatory first mutation and a second mutation at one of the other claimed positions in Taq.

13. Appellant I argued in essence that since document D2 was silent on determining the speed of the A3 mutant and since multiple reasons might be responsible for wt Taq's failure in producing PCR fragments in the presence of inhibitors (Tables 12 and 15), document D2 did not directly and unambiguously disclose the mutant Type-A DNA polymerases as defined in claim 1.

14. The board does not agree.

14.1 As set out above under claim construction, the conditions are not defined in claim 1 under which the polymerisation rate (i.e. speed) of the polymerase mutants is increased compared to wt Taq. This increase is thus a relative property since it suffices that an

increased speed is observed under one condition only. An example of this is shown in Figure 1 of post-published document D7 where the polymerisation rate of Taq mutants falling within the scope of claim 1 is comparable to that of wt Taq under "slow" cycling conditions, but increased under "fast" cycling conditions.

14.2 Tables 12 and 15 (part of Examples 5 and 6, respectively) of document D2 disclose that the A3 mutant generates PCR fragments in the presence of two inhibitors (high salt and phenol) while wt Taq produces no detectable PCR fragments. It was uncontested that the results in Tables 12 and 15 were obtained from experiments conducted under identical conditions.

14.3 Thus under identical conditions within a defined period of time the A3 mutant generates a PCR product contrary to wt Taq. Since the A3 Taq mutant was active under these conditions, there are no doubts that the mutant's polymerisation rate (i.e. the speed) was higher compared to wt Taq too, since wt Taq generated no detectable PCR product.

14.4 Appellant I's asserted issues of detection assay sensitivity and increased binding affinity of the A3 mutant are not convincing either. Firstly, the assay used is sensitive enough for detecting PCR fragments generated by the A3 mutant in the respective period of time, and secondly A3's high binding affinity for its template provides at best an explanation for the mechanism allowing a polymerisation in the presence of the inhibitors. However the polymerisation rate as defined in claim 1 concerns the relative incorporation of nucleotides per unit of time (claim construction above) irrespective of the mechanism underlying that

rate. Since the A3 mutant synthesised a PCR fragment, this enzyme necessarily incorporated nucleotides into a growing DNA strand during a specific period of time. Wt Taq did not generate such a PCR fragment in that time period. Compared to the A3 mutant, wt Taq has therefore necessarily a slower polymerisation rate under these conditions.

15. Since the higher resistance of A3 for polymerisation inhibitors shown in Tables 12 and 15 implies a faster polymerisation rate of this mutant compared to wt Taq under the conditions tested, document D2 anticipates the subject-matter of claim 1 at least under Article 54(3) EPC.

16. Auxiliary request 1 does not comply with the requirements of Article 54 EPC.

Auxiliary request 2

17. Claims 1 and 9 of auxiliary request 2 differ from the respective claims in auxiliary request 1 in that a disclaimer has been introduced to exclude the A3 mutant of document D2 from the subject-matter claimed. It is uncontested that this disclaimer is a so-called undisclosed disclaimer.

18. According to decision G 1/03 of the Enlarged Board of Appeal, published in OJ 2004, 413 (Headnote), an amendment to a claim by the introduction of a disclaimer may not be refused under Article 123(2) EPC for the sole reason that neither the disclaimer nor the subject-matter excluded by it from the scope of the claim have a basis in the application as filed. G 1/03 defines the criteria when such an undisclosed disclaimer is allowable, stipulating that it can be

introduced into a claim *inter alia* to restore novelty by delimiting a claim against the state of the art under Article 54(3) EPC but not under Article 54(2) EPC (except for a so-called accidental disclosure).

19. In order to determine whether document D2 is prior art under Article 54(2) or (3) EPC for the claimed subject-matter, it has to be assessed whether the subject-matter of claim 1 as a whole is entitled to claim priority from P and whether document D2 is entitled to claim priority from P1.

Priority

20. Document D2's priority based on P1 is valid for the reasons indicated above (point 10.3). Apart from the objections relating to formal entitlement to priority, appellant I has not raised further objections against the validity of document D2's priority claim.

Admittance of a new line of argument under partial priority

21. As regards the validity of the patent's priority claim based on P the following is noted. In reply to the communication under Article 15(1) RPBA indicating the board's preliminary opinion that priority was not valid for the subject-matter of claim 1 of auxiliary request 2, appellant I has submitted for the first time the argument that embodiments of claim 1 in reference to SEQ ID NO: 4 were entitled to claim a partial priority from P. During the discussion of this issue at the oral proceedings, appellant I requested not to admit under Article 13(2) RPBA appellant II's new line of argument under lack of partial priority and respective arguments under lack of novelty over document D2 for the

"comprising" embodiment in reference to SEQ ID NOs: 5 to 8 of claim 1.

22. Since appellant I introduced the issue of partial priority of certain embodiments of claim 1 in response to the board's communication shortly before the oral proceedings, appellant II could not have submitted their counterarguments on this issue earlier than at the oral proceedings. The board found merit in appellant I's arguments on partial priority of claim 1 and considered it a legitimate reaction to the board's preliminary opinion. For reasons of equity, the board therefore admitted into the proceedings the new lines of arguments on partial priority of both appellants. The same applied to appellant II's new line of argument under lack of novelty over document D2 in relation thereto.

The subject-matter of claim 1

23. It was uncontested that the claimed "consisting" and "comprising" embodiments of a mutant thermostable Type-A polymerase of claim 1 in reference to SEQ ID NO: 4 (points 1.5 and 1.6 above) are entitled to claim priority from P.

24. The parties were however in disagreement as to the entitlement to claim priority from P of the "consisting" and "comprising" embodiments of claim 1 in reference to SEQ ID NOs: 5 to 8.

25. Appellant I argued that claims 1, 2 and 10 including paragraphs [015], [024], [062] and Figure 1 of P disclosed the basis for these embodiments of claim 1.

25.1 The board does not agree.

25.2 In particular, the "*E507K*" mutation indicated in claim 2 of P which refers back to claim 1 is restricted to Taq due to the statement that "*the mutation is in Taq DNA polymerase*" (emphasis added). Since the E507K mutation is in Taq, this term cannot relate to any "*mutant DNA polymerase consisting of or comprising a mutation at a residue corresponding to residue 507 of wild-type Taq DNA polymerase*" (e.g. the polymerases encoded by SEQ ID NOs: 5 to 8) of P of which Taq is only one embodiment. The subject-matter of claim 10 of P being dependent on claim 1 does not change this fact because it is directed to mutations at other residues.

25.3 Paragraph [015] of P discloses "*exemplary DNA polymerases that can be mutated to create an engineered DNA polymerase according to the invention*" and Figure 1 of P discloses sequences of these wt polymerases. Since paragraph [015] and Figure 1 relate to wt polymerases only, no basis can be derived therefrom for the presence of a corresponding E507K mutation in the mutant DNA polymerases encoded by SEQ ID NOs: 5 to 8.

25.4 Also paragraph [024] of P provides no basis for these mutants in claim 1. This paragraph merely states that "*For example, the invention includes mutants of polymerases other than Taq DNA polymerase*" without, however, reporting on specific mutation(s) in these mutants. The opposition division held in the decision under appeal (point 23.2.6) that the last sentence of paragraph [024] ("*Thus, reference herein to specific mutations in wild-type Taq DNA polymerase can easily be correlated to corresponding mutations in other polymerases*") provided a basis for the E507K mutation in DNA polymerases other than Taq. The board does not agree because paragraph [024] of P is silent on any

specific mutation(s). This last statement in paragraph [024] represents therefore neither a link to the E507K mutation mentioned in claim 2 of P nor to the corresponding E507K mutation in SEQ ID NOs: 5 to 8 of claim 1. Accordingly, the corresponding E507K mutation for the polymerases encoded by SEQ ID NOs: 5 to 8 cannot be directly and unambiguously derived from paragraph [024] of P either.

25.5 Nor can such a disclosure be derived from paragraph [062] of P. This paragraph solely discloses Taq mutants without mentioning position 508 (contrary to claim 1) and, although the "*E507K mutation*" is disclosed, E507K is one of seven alternative mutations mentioned without being indicated as being particularly preferred.

25.6 The relevant date for the subject-matter of claim 1 in relation to the "consisting" and "comprising" embodiments concerning SEQ ID NOs: 5 to 8 is therefore the filing date of the patent application, while the "consisting" and "comprising" embodiments of claim 1 in relation to SEQ ID NO: 4 are entitled to claim a partial priority from P (G 1/15, published in OJ 2017, 82, Headnote).

26. Document D2 is thus prior art under Article 54(2) EPC for the subject-matter of claim 1 not enjoying priority, i.e. in relation to the "consisting" and "comprising" embodiments concerning SEQ ID NOs: 5 to 8, and prior art under Article 54(3) EPC for the subject-matter of claim 1 enjoying priority, i.e. in relation to the "consisting" and "comprising" embodiments concerning SEQ ID NO: 4.

27. The "comprising" embodiment in relation to SEQ ID NOs: 5 to 8 of claim 1 encompasses Type-A DNA polymerase

mutants that are structurally identical to Taq mutants disclosed in document D2. Appellant I has not argued to the contrary. The fact that the patent does not call these polymerases Taq mutants but gives them a different name does not change the fact that these mutants are structurally identical.

28. Since the undisclosed disclaimer added to claim 1 in auxiliary request 2 (point 17 above) thus removes embodiments of document D2 which belong to the state of the art pursuant to Article 54(2) EPC and are not an accidental disclosure, such amendment is not allowable under Article 123(2) EPC. Auxiliary request 2 comprises therefore added subject-matter (G 1/03, Headnote 2.1 and G 1/16, OJ 2018, 70, Headnote).

29. Auxiliary request 2 does not comply with the requirements of Article 123(2) EPC.

Auxiliary request 3

30. Claims 1 and 9 of auxiliary request 3 differ from the respective claims in auxiliary request 2 in their limitation to one reference sequence (SEQ ID NO: 4).

Admittance of auxiliary request 3 into the proceedings

31. Appellant II requested that auxiliary request 3 not be admitted into the proceedings, arguing in essence that appellant I did not provide any reasons in their SGA under Article 12(3) and (4) RPBA as to why this auxiliary request (filed as auxiliary request 5 with the SGA) overcame any of the objections raised. In similar circumstances these provisions had led the Boards to decide not to admit auxiliary requests, for example, in case T 559/20.

32. It is uncontested that present auxiliary request 3 has been submitted already during the opposition proceedings and was merely re-submitted with appellant I's SGA. Appellant I stated in this context (SGA, item 6) that: "*All of these Requests meet the requirements of clarity, enablement and inventive step as set forth for the allowed AR2 above and the requirements of added matter and novelty as explained for at least one of the MR and the AR1 or AR2 above*". A similar statement is found in their letter of 14 December 2022 (section 2.2), filed in response to appellant II's reply to appellant I's SGA.

33. The board agrees with appellant I that the purpose of the amendment by deletion of all reference sequences except for SEQ ID NO: 4 in claims 1 and 9 of auxiliary request 3 was straightforward, as it was clear that it was made to establish novelty and to overcome the finding of added subject-matter. In these circumstances and differently from the situation present in the case law cited by appellant II, the level of substantiation provided in the SGA, as well as in the further submission of 14 December 2022, is considered appropriate. Further, as this amendment is occasioned by objections raised in the proceedings, it complies with the requirements of Rule 80 EPC.

34. Furthermore the opposition division held that the patent in suit could be maintained in amended form on a request ranking higher than present auxiliary request 3 (section III above). Since appellant I was thus not negatively affected as regards auxiliary request 3, also for this reason the situation in the present case differs fundamentally from that underlying T 559/20, wherein the set of claims in suit had been the object

of the appealed decision (T 559/20, Reasons 3.2). Hence, contrary to appellant II's arguments, decision T 559/20 cannot therefore support their case either.

35. Auxiliary request 3 was thus admitted into the appeal.

Added subject-matter, extension of scope and clarity

36. Appellant II has not submitted any objections against auxiliary request 3 under added subject-matter and clarity and the board has none either.

37. In essence, amended claims 1 and 9 find a basis in claims 1 and 13 as filed. Further the introduced disclaimer in claims 1 and 9 does not comprise added subject-matter since it is in line with the criteria set up in G 1/03 (Headnote, supra). Moreover, due to the limitation of claims 1 and 9 to the mutant polymerases encoded by SEQ ID NO: 4 and the introduction of the disclaimer, the scope of protection conferred by the subject-matter of these claims is more restricted than that of the respective claims as granted. Since claims 1 and 9 have been limited to embodiments that were already comprised in the respective claims as granted and the disclaimer is clear, no issues under Article 84 EPC arise.

38. Auxiliary request 3 complies therefore with the requirements of Articles 123(2), (3) and 84 EPC.

Sufficiency of disclosure

39. Article 83 EPC requires that the application discloses the invention in a manner sufficiently clear and complete for it to be carried out by the skilled person. The claimed invention must be sufficiently

disclosed on the filing date (Case Law of the Boards of Appeal of the EPO, 10th edition 2022, ("Case Law"), II.C.2.) based on the application as a whole (Case Law, II.C.3.1), in consideration of the common general knowledge of the skilled person (Case Law, II.C.4.1.). While at least one way of carrying out the claimed invention must be disclosed, this disclosure is sufficient only if it allows the invention to be performed over substantially the whole range claimed (Case Law, II.C.5.2., II.C.5.4 and II.C.7.1.2). In application of the principle that each of the parties to the proceedings carries the burden of proof for the facts they allege, the weight of the submissions required in opposition proceedings to rebut the argument that the patent meets the requirements of sufficiency of disclosure depends on the strength of the teaching contained in the patent application, eventually on account of the common general knowledge.

40. The application as filed discloses three exemplary Taq mutants "*2C2*", "*Taq42*" and "*3B*" including their sequences (SEQ ID NOs: 38, 36 and 28, respectively) and the Taq wt sequence (SEQ ID NO: 4) for generating further mutants. All three mutants "*2C2*", "*Taq42*" and "*3B*" are modified at position 507 from E to K (E507K) and at least at one of the seven other positions indicated in claim 1. It is uncontested that these exemplified Taq mutants show the claimed functional properties (point 1.3 above).

40.1 Furthermore the application as filed discloses a method for generating Taq mutants *de novo* (Example 1), assays for selecting mutants showing a faster polymerisation rate compared to wt Taq (Examples 2 and 3), and several assays for testing the resistance of Taq and its mutants to various inhibitors (Examples 4 to 9).

40.2 In addition, the application as filed teaches that the mutation at E507 to K generates Taq mutants with an enhanced polymerisation speed compared to wt Taq. Moreover it is taught that the E507K mutation in combination with other mutations at the positions indicated in claim 1 generates Taq mutants having an enhanced polymerisation speed and inhibitor resistance compared to wt Taq (paragraph [055]). This is supported by experimental data of the mutants "*2C2*", "*Taq42*" and "*3B*" compared to a E507K only mutant ("*5A2*") and wt Taq (Example 6, paragraphs [094] to [098], Figures 6 and 7).

40.3 It is uncontested that further Taq mutants comprising E507K and at least one additional amino acid substitution at a position indicated in claim 1 can be made by standard means. It is also uncontested that the assays mentioned in the application as filed can be used for testing candidate polymerase mutants for the claimed functional properties.

41. Appellant II argued however that the large amount of potential mutants to be generated and tested falling within the scope of claim 1 amounted to undue burden. Moreover, these mutants comprised necessarily non-working embodiments for structural reasons.

41.1 This is not convincing. Appellant II - who, in view of the above summarised teaching in the patent, bears the burden of proof for their objection, has not submitted a single non-working embodiment of the claimed mutant DNA polymerases or provided any other verifiable fact that not substantially all mutants falling within the scope of claim 1 show the claimed functional

properties. The fact that the scope of claim 1 is broad is by itself irrelevant for sufficiency of disclosure.

41.2 Even if the properties as defined in claim 1 might not be achieved by some Taq mutants falling within the claimed scope, this is immaterial for sufficiency of disclosure.

41.3 In this context the board concurs with appellant I's argument that it must be kept in mind that the standard of a direct and unambiguous disclosure under Article 123(2) EPC is different, namely stricter, than that of a sufficient disclosure under Article 83 EPC. In order to meet the latter requirement, it is sufficient that the skilled person can reproduce the invention without making use of non-available teachings and without becoming inventive. An invention is sufficiently disclosed even if specific instructions are lacking of how to obtain substantially all possible compound variants encompassed by a claimed functional definition, as long as there are suitable variants known to the skilled person through the disclosure or common general knowledge, which provide the same effect for the invention (T 32/84, Headnotes I and II and T 292/85, Reasons 3.1.5).

41.4 In the absence of any evidence to the contrary, the board has no doubts that the teaching of the application as filed (points 40 and 40.1 to 40.3 above) enables the skilled person to find further mutants showing the claimed properties across substantially the whole scope claimed without undue burden (application as filed, paragraph [052] and Case Law, II.C.5.4).

42. Appellant II further argued in essence that because the application as filed provided no evidence that the

E507K mutation in Taq in combination with a single further mutation at a position indicated in claim 1 generated Taq mutants having the desired functional properties, it was not credible that all mutant combinations achieved the technical effects. According to established case law, post-published evidence (document D7) could thus not be relied on for confirmatory purposes (G 2/21, in OJ 2023, 85, Reasons 74 and 77, T 2790/17, Reasons 2.3 and 2.8.5). Nor disclosed the application as filed a technical concept how the at least two mutations in Taq achieved the claimed functional properties or any other guidance that helped the skilled person finding substitutions that resulted in Taq mutants with the claimed properties. Moreover the lack of guidance in the application as filed amounted to undue burden (T 25/20 and T 2164/21). This was supported by documents D1 and D3 which disclosed that Taq mutants, although having mutations at the required positions, did not have the claimed properties.

43. These arguments are not persuasive.

43.1 In G 2/21 the Enlarged Board of Appeal ruled that in order to meet the requirements of Article 83 EPC in a second medical use claim, the proof of a claimed therapeutic effect has to be provided in the application as filed, in particular if, in the absence of experimental data in the application as filed, it would not be credible to the skilled person that the therapeutic effect is achieved. A lack in this respect cannot be remedied by post-published evidence (Reasons 77).

43.2 Contrary to appellant II's arguments, the application as filed discloses experimental evidence (Figures 6 and

7 in conjunction with Example 6, paragraphs [094] to [098]) that the claimed properties resulting from the claimed mutations in Taq are at least credible over the whole scope claimed. The three exemplary Taq mutants ("*2C2*", "*Taq42*" and "*3B*") disclosed in the application as filed all contain the compulsory E507K and three or six additional amino acid substitutions at positions indicated in claim 1. These 4-fold and 7-fold Taq mutants show the claimed functional properties when compared to a E507K only Taq mutant ("*5A2*", i.e. a 1-fold mutant) and wt Taq. This experimental finding is moreover consistent with the general teaching in the application as filed (paragraph [055]) that the E507K mutation increases the mutants' polymerisation rate and that the other mutations indicated in claim 1 increase the mutants' inhibitor resistance compared to wt Taq.

43.3 Based on these data in the application as filed and in the absence of any evidence to the contrary (for which appellant II bears the burden of proof), the board has no doubts that also 2-fold or 3-fold mutants falling within claim 1 show the claimed properties. Hence, these properties are credible based on the data in the application as filed alone. For that reason, post-published data can be taken into account for confirmatory purposes (CLBA, II.C.7.2.2.b)). All findings reported in the application as filed are confirmed in post-published document D7 (e.g. abstract, and page 5, right column, second paragraph to page 7, right column, first paragraph).

43.4 The factual situation in this case fundamentally differs thus from that underlying decision T 2790/17, relied on by appellant II, wherein the competent board found that Example 5 of the respective application did not provide sufficient evidence that increasing GBA

activity led to lowered alphaS levels as required for the therapeutic application claimed. The application as filed in that case even disclosed evidence to the contrary (the level of alphaS increased in the presence of GBA, Reasons 2.5 and 2.6.6). The same applies to the case underlying decision T 25/20, also cited by appellant II, where all parties agreed that the application as filed did not contain any experimental evidence in relation to the therapeutic effect in question. Nor was a teaching disclosed why a certain agent was at all suitable for the claimed therapeutic application (Reasons 5 and 6.4).

43.5 Similar considerations apply to decision T 2164/21. Here the application as filed disclosed in the sole working example a replacement of one amino acid by 18 different amino acids in an antigen binding site in a single antibody. It was shown that some of these amino acid replacements resulted in antibodies that no longer bound to the antigen while others retained this property (Reasons 39 and 40). The competent board held that in the presence of serious doubts arising from these facts and in the absence of any guidance about a general concept in the application as filed, a concept that even went against established expectations in that field, the skilled person faced undue burden (Reasons 39, 40, 42 to 45, 53 and 55). In the present case, however, the application as filed discloses experimental evidence of the claimed functional properties, so that in the absence of facts concerning a single non-working Taq mutant falling within the scope claim 1, appellant II's submissions are speculative only.

43.6 As regards document D1, this document discloses several Type-A DNA polymerases which contain the E507K mutation

and at least one additional mutation at V155I and/or E734N (e.g. SEQ ID NOs: 66 and 374) including several other mutations not mentioned in claim 1, including D785N. As regards the functional properties of these mutant polymerases, document D1 consistently states that they have a "*reduced or absent synthetic activity*" (e.g. paragraph [0247]), which implies that these mutants show a reduced or even lacking polymerisation rate too compared to their respective wt polymerases (point 14.4 above). In particular, document D1 discloses that the D785N destroys the polymerase activity of the mutants (paragraph [0761]). Document D1 thus discloses polymerases that because of a deliberately introduced D785N mutation either lack or have a low polymerisation rate only. The presence of mutations at E507K, V155I and/or E734N (i.e. of mutations mentioned in claim 1) cannot remedy that fact. Conclusions on the effect of the claimed mutations on the polymerisation rate can therefore not be drawn from document D1's teaching.

43.7 As regards document D3, this document discloses only polymerase mutants which contain E507K but none of the other mutations mentioned in claim 1. Since the mutants in document D3 do not even comply with the minimal structural requirements of the claimed polymerases (i.e. mutation E507K plus at least one further mutation at positions selected from residue selected from 59, 155, 245, 375, 508, 734, and 749), no conclusion on the functional properties of the claimed mutations can be drawn from document D3 either.

44. Appellant II further argued that the skilled person was faced with undue burden because due to the high number of mutants falling within the "comprising" embodiment of claim 1, the skilled person was unable to ascertain

whether he/she was working within the limits of the claim. This is not persuasive either since this objection rather concerns an issue of clarity and not one of sufficiency. However, because the subject-matter of claim 1 is an embodiment of claim 1 as granted, compliance with the requirements of Article 84 EPC is not to be examined for this subject-matter (G 3/14 published in OJ 2015, 102, Headnote).

45. In view of the considerations above, the board concludes that the application as filed discloses the invention as defined in claim 1 in a manner sufficiently clear and complete for it to be carried out by the skilled person. Auxiliary request 3 complies with Article 83 EPC.

Novelty

Admittance of a new line of argument under lack of novelty of document D2 against the subject-matter of claim 7

46. Appellant II submitted with their SGA that the specific Taq mutants characterised by SEQ ID NOs: 20 and 22 of claim 7 of auxiliary request 2 lacked novelty over the mutants disclosed in Table 2 and claim 23 of document D2. Since the subject-matter of claim 7 of auxiliary request 3 is identical to that of auxiliary request 2, this objection applies to this request as well.

46.1 Claim 7 of auxiliary request 3 is identical to claim 7 as granted (main request). It is uncontested that appellant II did not raise this objection during the opposition proceedings, but rather has submitted it for the first time in appeal proceedings.

46.2 In view of this course of events, appellant II's argument under lack of novelty against claim 7 of auxiliary request 3 is new to the proceedings and represents an amendment of their case (Article 12(4) RPBA). Since this argument should have been submitted earlier and appellant II has not provided any justification, neither with their SGA nor with their reply to appellant I's SGA, as to why it has only been raised in appeal, the board decided not to admit this line of argument into the appeal proceedings (Article 12(4) RPBA).

Novelty over documents D1 and D4

47. Appellant II submitted that the mutant Type-A DNA polymerases of claim 1 were anticipated by the disclosure of documents D1 and D4.

48. As regards document D1, this document as indicated above under sufficiency of disclosure discloses several Type-A DNA polymerases that contain the E507K mutation and at least one additional mutation at one of the other positions indicated in claim 1, including the D785N mutation which destroys their polymerase activity (paragraph [0761]). Since this implies that the polymerisation rate of these mutants is necessarily lower than that of their respective wt polymerases (point 14.4 above), appellant II's argument is not convincing. This applies to all mutants referred to by appellant II (SGA, Table on pages 23 and 24) because detailed structural information about these mutants, in particular about the presence of the E734N mutation, are not disclosed in document D1. However in light of document D1's general aim in generating polymerase mutants having a reduced polymerisation activity (paragraph [0066]), the board agrees with the

opposition division (decision under appeal, point 24.2.3 and 24.2.5) that document D1 does not directly and unambiguously disclose polymerase mutants falling within the scope of claim 1.

49. As regards document D4, this document is silent on Taq mutants carrying the E507K mutation and at least one further mutation selected from any of the other positions indicated in claim 1. Document D4 instead discloses Taq mutants at positions 626, 706, 707 and 708, for example, E708K in a Taq polymerase named KT 10 (page 3, right column, second to fourth paragraph). The E708K mutation in KT 10 has the effect that the polymerase "*remained functional in at least 10-15% blood*", i.e. it made the polymerase resistant against blood-based inhibitors when compared to wt Taq (page 4, left column, second paragraph).

49.1 Appellant II argued that since the Taq mutants of the "comprising" embodiment of claim 1 were devoid of any framework, the claimed mutants were anticipated by the KT 10 mutant of document D4.

49.2 The board does not agree. The Taq mutants of the "comprising" embodiment in claim 1 are not devoid of any framework as regards the positions which have to be mutated. On the contrary, claim 1 defines the positions in reference to the Taq wt sequence SEQ ID NO:4 which represents the framework (reference) against which corresponding amino acids have to be replaced. The E708K mutation in the KT 10 Taq mutant of document D4 cannot therefore anticipate the E507K mutation indicated in claim 1 since both mutations are at completely different positions in the amino acid sequence of Taq.

50. The subject-matter of claim 1 is thus novel over the disclosure of documents D1 and D4.

51. Auxiliary request 3 complies with Article 54 EPC.

Inventive step

52. Appellant II submitted that either document D2 or D3 represented the closest prior art. Since document D2 is prior art under Article 54(3) EPC for the subject-matter claimed (point 26 above), D2's teaching is excluded from the assessment of inventive step.

Closest prior art

53. Appellant I requested not to admit a line of argument under inventive step based on document D3 as closest prior art. The board decided, however, that this line of argument was not new and was thus to be taken into consideration. In view of the conclusions on inventive step (see below), no further reasoning as to the admission of this line of argumentation is needed in the present decision.

54. Appellant I moreover argued that, in agreement with the decision of the opposition division, document D3 was not a suitable starting point for the discussion of inventive step. Again the board disagrees, but in view of the conclusions reached, no justification for this finding is needed other than noting that document D3 has been selected by appellant II (then opponent) as the closest prior art: since an inventive step can only be acknowledged if the claimed subject-matter is not obvious having regard to any prior art, it has to be shown that this is the case over document D3's teaching as well.

55. It is uncontested that document D3 is silent on Taq polymerases or chimeric enzymes consisting of Taq and Tth polymerases containing mutations that improve (1) the enzymes' resistance to polymerase inhibitors and (2) their polymerisation rate compared to wt Taq.

56. The chimeric polymerases of document D3 have been generated to distinguish particular RNAs from closely related molecules (page 10, lines 20 to 23). This purpose is achieved by generating various Taq mutants having an improved 5' nuclease activity, for example, the parent mutant "*W417L/G418K/E507Q/H784A*" termed "*Taq 4M*" (page 134, lines 3 to 8). Starting from there various further mutant enzymes have been generated showing an even improved RNA target-dependent activity, some of which include the E507K mutation. However none of these mutants contains the E507K mutation and at least one further mutation selected from the positions indicated in claim 1.

Technical problem

57. The claimed Type A DNA polymerase mutants differ thus from those in document D3 in that they contain in addition to the E507K mutation at least one further mutation at a residue selected from positions 59, 155, 245, 375, 508, 734, 749 of wt-Taq DNA. These at least two mutations must provide mutants with a faster polymerisation rate and a higher resistance to inhibitors compared to wt Taq.

58. While the patent shows that mutants falling within the claimed scope have increased polymerisation rate and higher resistance to inhibitors, document D3 is, as set out above in point 55, silent on any mutations

affecting the mutant polymerases' polymerisation rate and inhibitor resistance.

59. In the absence of comparative examples the board cannot establish whether the effects caused by the at least one additional mutation at the claimed positions in Taq are superior to potential effects caused by mutations in the Taq polymerases disclosed in document D3.

60. Accordingly, the technical problem to be solved resides in the provision of alternative mutant thermostable Taq polymerases having an increased polymerisation rate and resistance against inhibitors of the polymerase activity compared to wt Taq.

61. The subject-matter of claim 1 solves this problem.

Obviousness

62. Not only is document D3 silent on mutations potentially affecting the polymerisation rate and inhibitor resistance of Taq (point 55 above), it also provides no pointers on which other positions wt Taq might be mutated to arrive at alternative mutants having an increased polymerisation rate and inhibitor resistance.

63. Thus the skilled person starting from document D3 and faced with the technical problem defined above would not have been directed in an obvious manner to introduce a mutation in wt Taq at least at one of the specific positions indicated in claim 1, except for E507K. Accordingly, the subject-matter of claim 1 is inventive over the teaching of document D3. Analogous considerations apply for the subject-matter of claims 8, 9 and 14.

64. Auxiliary request 4 complies with 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 opposition division with the order to maintain the patent on the basis of the claims of auxiliary request 3, filed as auxiliary request 5 with the statement of grounds of appeal, drawings: figures 1 to 10D of the specification and a description to be adapted thereto, if needed.

The Registrar: The Chair:

C. Rodríguez Rodríguez T. Sommerfeld

Decision electronically authenticated