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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: NUCLEIC ACIDS AND PROTEINS WITH THIOREDOXIN REDUCTASE ACTIVITY

(57) Abstract: The present invention relates to the use of a variety of methods for generating functional thioredoxin reductase variants in which at least one physical, chemical or biological property of the variant is altered in a specific and desired manner when compared to the wild-type protein.

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International application No.
PCT/US02/14358

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 9/00; 15/00; C12P 21/04; C07H 21/04, 21/02; C07K 1/00
US CL : 435/183, 71.1, 440; 536/23.2, 23.1; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/183, 71.1, 440; 536/23.2, 23.1; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
thioredoxin, mutant, substrate, nadh, nadph

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 6,380,372 B1 (CHO et al) 30 April 2002 (30.04.2002), entire document.	1-29, 34 and 36-66
Y	WO 00/58352 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 05 October 2000 (05.10.2000), entire document.	1-29, 34 and 36-66
Y	RUSSEL. M. Sequence of thioredoxin reductase from Escherichia coli. Relationship to other flavoprotein disulfide oxidoreductases. J Biol Chem. June 1988 Vol 263. No. 18, pages 9015-9019.	1-28, 34 and 36-66
Y	JACQUOT. J.P. Arabidopsis thaliana NAPHP thioredoxin reductase. cDNA characterization and expression of the recombinant protein in Escherichia coli.J Mol Biol. January 1994, Vol 235. No. 4, abstract.	1-29 and 36-66

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 September 2004 (29.09.2004)	20 OCT 2004

Name and mailing address of the ISA/US	Authorized officer
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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-29,34 and 36-66 (thioredoxin derived from E.coli and A. thaliana)
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-29 and 36-66, drawn to a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *E. coli*.

Group II, claim(s) 1-28, 30 and 36-66, drawn to a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *B. subtilis*.

Group III, claim(s) 1-28, 31 and 36-66, drawn to a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *M. leprae*.

Group IV, claim(s) 1-28, 32 and 36-66, drawn to a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *S. cerevisiae*.

Group V, claim(s) 1-28, 33 and 36-66, drawn to a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *N. crassa*.

Group VI, claim(s) 1-28, 34 and 36-66, drawn to a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *A. thaliana*.

Group VII, claim(s) 1-28, 35 and 36-66, drawn to a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *H. sapien*.

Group VIII, claim(s) 67-73, drawn to a method of producing a plant with a modified thioredoxin reductase.

Group IX, claim(s) 74-77, drawn to a method of making oil bodies comprising a modified thioredoxin reductase.

The inventions listed as Groups I-IX do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking Groups I-IX appears to be that they all relate to a modified thioredoxin reductase.

However, Mulrooney et al. teaches modified thioredoxin reductase (abstract).

Therefore, the technical feature linking the inventions of Groups I-IX does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art. Futher, Groups I-VII are drawn to various thioredoxin scaffold proteins comprising proteins derived from different sources. These scaffold proteins have different structure and function, such as substrate and cofactor specificity, and therefore do not share any special technical feature.

The special technical feature of Group I is a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *E. coli*.

The special technical feature of Group II is a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *B. subtilis*.

The special technical feature of Group III is a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *M. leprae*.

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The special technical feature of Group IV is a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *S. cerevisiae*.

The special technical feature of Group V is a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *N. crassa*.

The special technical feature of Group VI is a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *A. thaliana*.

The special technical feature of Group VII is a method of altering cofactor specificity for a thioredoxin reductase scaffold protein derived from *H. sapien*.

The special technical feature of Group VIII is a plant with a modified thioredoxin reductase.

The special technical feature of Group IX is a method of making oil bodies comprising a modified thioredoxin reductase.