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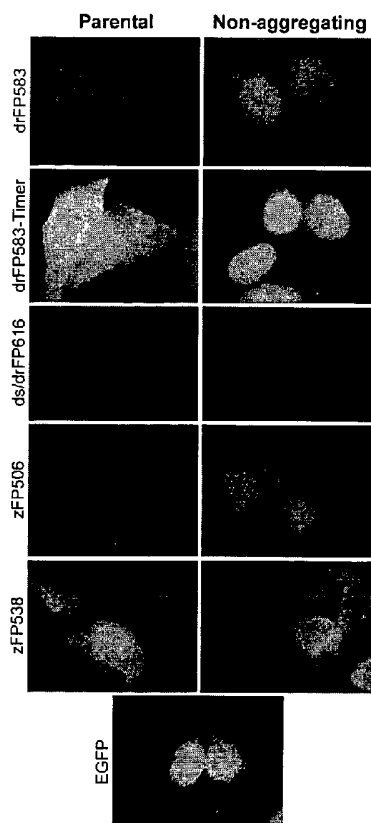
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[Continued on next page]

(54) Title: NON AGGREGATING FLUORESCENT PROTEINS AND METHODS FOR USING THE SAME



(57) Abstract: Nucleic acid compositions encoding non-aggregating chromo/fluoroproteins and mutants thereof, as well as the proteins encoded by the same, are provided. The proteins of interest are polypeptides that are non-aggregating colored and/or fluorescent proteins, where the non-aggregating feature arises from the modulation of residues in the N-terminus of the protein and the chromo and/or fluorescent feature arises from the interaction of two or more residues of the protein. Also provided are fragments of the subject nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compositions find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compositions, are provided.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,  
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent  
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,  
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**Published:**

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27 November 2003

INTERNATIONAL SEARCH REPORT

International Application No  
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<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC 7 C12N15/65 C07K14/435 C07K16/18 C12N5/10 A01K67/00 C12Q1/68		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N C07K A01K C12Q		
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 practical, search terms used) SEQUENCE SEARCH, MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WETZEL RONALD: "Mutations and off-pathway aggregation of proteins." TRENDS IN BIOTECHNOLOGY, vol. 12, no. 5, 1994, pages 193-198, XP001093961 ISSN: 0167-9430 the whole document ---	1-13, 16-20
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in 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 document but 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  6 August 2002		Date of mailing of the international search report  23/08/2002
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  Herrmann, K

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/05749

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>BAIRD GEOFFREY S ET AL: "Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 97, no. 22, 24 October 2000 (2000-10-24), pages 11984-11989, XP002208898 October 24, 2000 ISSN: 0027-8424 page 11989, right-hand column, paragraph 2 ---</p>	1-13, 16-20
Y	<p>WALL MARK A ET AL: "The structural basis for red fluorescence in the tetrameric GFP homolog DsRed." NATURE STRUCTURAL BIOLOGY, vol. 7, no. 12, December 2000 (2000-12), pages 1133-1138, XP001095366 ISSN: 1072-8368 the whole document ---</p>	1-13, 16-20
A	<p>MATZ MIKHAIL V ET AL: "Fluorescent proteins from nonbioluminescent Anthozoa species." NATURE BIOTECHNOLOGY, vol. 17, no. 10, October 1999 (1999-10), pages 969-973, XP002208899 ISSN: 1087-0156 figure 1 ---</p>	1-13, 16-20
A	<p>FRADKOV ARKADY F ET AL: "Novel fluorescent protein from Discosoma coral and its mutants possesses a unique far-red fluorescence." FEBS LETTERS, vol. 479, no. 3, 2000, pages 127-130, XP002208900 ISSN: 0014-5793 figure 1 ---</p>	1-13, 16-20
A	<p>WIEHLER JENS ET AL: "Mutants of Discosoma red fluorescent protein with a GFP-like chromophore." FEBS LETTERS, vol. 487, no. 3, 2001, pages 384-389, XP002208901 ISSN: 0014-5793 page 387, left-hand column, paragraph 3 page 388, left-hand column, last paragraph -right-hand column, paragraph 1 ---</p>	1-13, 16-20

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INTERNATIONAL SEARCH REPORT

Inter: I Application No  
PCT/US 02/05749

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>YANUSHEVICH YURII G ET AL: "A strategy for the generation of non-aggregating mutants of Anthozoa fluorescent proteins." FEBS LETTERS, vol. 511, no. 1-3, 30 January 2002 (2002-01-30), pages 11-14, XP002208902 30 January, 2002 ISSN: 0014-5793 the whole document ---</p>	1-13, 16-20
P, X	<p>WO 01 27150 A (FRADKOV ARCADY FEDOROVICH ; LABAS YULII ALEKSANDROVICH (RU); LUKYAN) 19 April 2001 (2001-04-19) page 19, line 9 -page 21, line 10 -----</p>	11

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 02/05749

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

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

Claim 13 also encompasses transgenic human beings. This subject-matter is contrary to morality in certain PCT member states, e.g. Art. 53(a) of the EPC.
2.  Claims Nos.: 14, 15 and partially claims 1-13, 16-18  
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:  

see FURTHER INFORMATION sheet PCT/ISA/210
3.  Claims 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:

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.:
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.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

Continuation of Box I.2

Claims Nos.: 14, 15 and partially claims 1-13, 16-18

Claims 14 and 15:

Claims 14 and 15 fail to comply with the requirements of Art. 6 PCT (clarity) to such an extent that a meaningful search could not be carried out (Art. 17(2)(a)(ii) PCT). The wording "in an application..." and "the improvement comprising..." does not make sense.

Claims 1-13 and 16-18 (all partially):

Claims 1-9, 12, 13 and 16-18 cover or refer to all nucleic acids having the desired characteristic of (i) encoding a non-aggregating, chromo- or fluorescent protein and (ii) being a mutant of an aggregating Cnidarian chromo- or fluorescent protein. However, the application provides support (Art. 6 PCT) and disclosure (Art. 5 PCT) for only a limited number of such nucleic acids. Thus, a meaningful search over the whole of the scope claimed is impossible. Consequently, the search has been limited to a nucleic acid encoding a non-aggregating, chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein wherein a basic residue located near the N-terminus of said aggregating protein has been substituted for a neutral residue (also cf. present claim 19 and the abstract of Yanushevich et al., cited here as an expert's opinion).

The same holds true for the subject-matter of claims 10 and 11. The search has been limited to a non-aggregating, chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein wherein a basic residue located near the N-terminus of said aggregating protein has been substituted for a neutral residue.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Intern. Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0127150      A	19-04-2001	AU      1086701 A	23-04-2001
		WO      0127150 A2	19-04-2001
		WO      0034326 A1	15-06-2000
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		WO      0034322 A1	15-06-2000
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		WO      0034325 A1	15-06-2000

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