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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Published:

— *with international search report (Art. 21(3))*

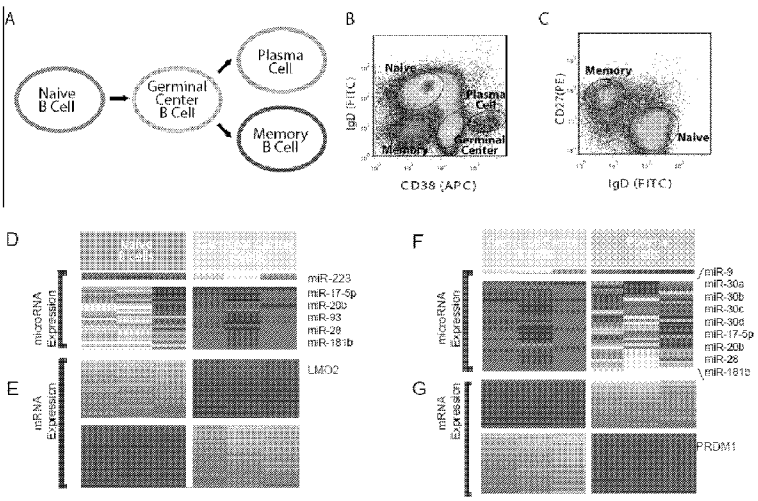
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) **Date of publication of the international search report**:
28 July 2011

(54) **Title**: MICRORNA AND USE THEREOF IN IDENTIFICATION OF B CELL MALIGNANCIES

Figure 1



(57) **Abstract**: Disclosed are nucleic acid sequences, including microRNA sequences and cDNA sequences, as well as vectors, DNA libraries, microarrays, and recombinant cells comprising the nucleic acid sequences described herein. Methods of determining the B cell stage from which a B cell malignancy is derived. Methods of identifying B cell malignancies are also provided. Methods of diagnosing B cell malignancies are provided. Such methods comprise, in certain embodiments, detecting one or more microRNAs or cDNAs as disclosed herein.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/58952

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C07H 21/04 (2011.01)
USPC - 435/6; 536/24.31
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)
USPC: 435/6; 536/24.31

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)
Electronic data bases: PubWEST (PGPB, EPAB, JPAB, USPT); Google Scholar: B-cell lymphoma (e.g. diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), follicular lymphoma, Hodgkin's lymphoma (HL), Burkitt's lymphoma) , microRNA (miRNA), antagomir, antagonist, anti-mIR. Locked nucleic acid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0143326 A1 (OBAD et al.) 4 June 2009 (04.06.2009) SEQ ID NO: 739, para [0003], pg 60 table 1	1-10
A	US 2006/0199233 A1 (DAHLBERG et al.) 7 September 2006 (07.09.2006) abstract, para [0012]-[0018]	1-10

☐ Further documents are listed in the continuation of Box C. ☐

* 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 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	Date of mailing of the international search report
17 May 2011 (17.05.2011)	0 2 JUN 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore 6.3 SEQ ID NO: 763

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/58952

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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:

2. ☐ Claims 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. ☒ Claims Nos.: 11-16, 38
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
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.

Group I+: claims 1-10, drawn to an isolated nucleic acid molecule. The first invention is restricted to SEQ ID NO: 763. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. Due to the number of sequences in this application, an additional invention(s) of Group I+ will be defined as necessary depending on Applicant's ultimate payment of additional fees. The additional sequences will be searched if applicant pays for each additional sequence or shows that the sequences share a special technical feature, i.e. a common structure or feature that defines a contribution over the prior art. Note that each additional sequence to be searched must be specified by the Applicant in the response to this invitation and must either (1) have an additional invention fee paid or (2) have a showing that the sequences share a common structure or feature that defines a contribution over the prior art.
----------continuation on Extra 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 additional fees, this Authority did not invite payment of additional fees.

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.:
Claims 1-10 limited to SEQ ID NO: 763

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

********** Supplemental Box **********

continuation of Box III (Lack of Unity of Invention):

Group II+, claims 17-31, drawn to a method of determining the B cell stage of a B cell malignancy in a subject by determining the level of expression of at least one micro RNA in a sample comprising a B cell taken from the subject relative the level of expression of the at least one microRNA in a control sample. The first invention is restricted to hsa-let-7a, the first entry of Table 4. Should an additional fee(s) be paid, Applicant is invited to elect an additional microRNA(s) to be searched. The exact claims searched will depend on the specifically elected microRNA(s) to be searched.

Group III+, claims 32-37, 39-41, drawn to a micro array comprising miRNA-specific probe oligonucleotides. The first invention is restricted to SEQ ID NO:763. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. The exact claims searched will depend on the specifically elected SEQ ID NO(s). Due to the number of sequences in this application, an additional invention(s) of Group III+ will be defined as necessary depending on Applicant's ultimate payment of additional fees. The additional sequences will be searched if applicant pays for each additional sequence or shows that the sequences share a special technical feature, i.e. a common structure or feature that defines a contribution over the prior art. Note that each additional sequence to be searched must be specified by the Applicant in the response to this invitation and must either (1) have an additional invention fee paid or (2) have a showing that the sequences share a common structure or feature that defines a contribution over the prior art.

The inventions listed as Groups I+ through III+ 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:

Groups I+ and III+ do not include the inventive concept of a a method of determining the B cell stage of a B cell malignancy in a subject by determining the level of expression of at least one micro RNA in a sample comprising a B cell taken from the subject relative the level of expression of the at least one microRNA in a control sample, as required by Group II+.

Groups I+ and II+ do not include the inventive concept of a micro array comprising miRNA-specific probe oligonucleotides, as required by Group III+.

The inventions of Group II+ share the technical feature of a method of determining the B cell stage of a B cell malignancy in a subject by determining the level of expression of at least one micro RNA in a sample comprising a B cell taken from the subject relative the level of expression of the at least one microRNA in a control sample. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2006/0199233 A1 to DAHLBERG et al. (hereinafter "Dahlberg") that teaches a method of identifying and diagnosing a B cell malignancy in a subject comprising determining the level of expression of at least one microRNA in a sample comprising a B cell taken from the subject relative the level of expression of the at least one micro RNA in a control sample, wherein the at least one microRNA is selected from the microRNAs listed in Table 4 (para [0012]; "methods for diagnosing B-cell lymphoma in an animal comprising the step of assaying a B-cell sample isolated from the animal to determine the amount ofmiR-155 in the sample, and diagnosing B-cell lymphoma in the animal if the amount of miR-155 in the B-cell sample is higher than the amount of miR-155 in normal B-cells"). As said method was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Group III+ share the technical feature of a micro array comprising miRNA-specific probe oligonucleotides. However, this shared technical feature does not represent a contribution over the prior art as being anticipated by WO2007/1122754 A2 (ELMAN) that discloses such a miRNA microarray useful for analysing LNA antago-mir knock-down specificity (pg 74, Example 8). As said array was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another technical feature of the inventions listed as Groups I+ and III+ is the specific nucleic acid sequence recited therein. The inventions do not share a special technical feature, because US 2009/0143326 A1 to OBAD et al. (hereinafter "Obad") discloses the claimed SEQ ID NOs: 763 (pg 60 table 1 SEQ ID NO: 739 [i.e. miR-548o] in Table 1 is 22 nucleotides in length and comprises 100% sequence homology to application SEQ ID NO: 763). Without a shared special technical feature, the inventions lack unity with one another.

Groups I+, II+, and III+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.