

# **DETECTING HEMATOLOGICAL DISORDERS USING CELL-FREE DNA IN BLOOD**

## **CROSS-REFERENCES TO RELATED APPLICATIONS**

**[0001]** The present application claims priority from and is a nonprovisional application of  
5 U.S. Provisional Application No. 62/343,050, entitled “Detecting Hematological Disorders  
Using Cell-Free DNA In Blood” filed May 30, 2016, the entire contents of which are herein  
incorporated by reference for all purposes.

## **BACKGROUND**

**[0002]** To determine whether a hematological disorder (e.g., anemia) exists in a person,  
10 conventional techniques perform a histological examination of a bone marrow biopsy.  
However, a bone marrow biopsy is an invasive procedure leading to pain and anxiety for  
patients undergoing such a procedure. Therefore, it is desirable to identify new techniques to  
detect and characterize hematological disorders in a person.

**[0003]** Anemia can be caused by multiple clinical conditions, each with its own treatment.  
15 Hence, it would be clinically useful to ascertain the cause of a case of anemia, and then  
further investigate or treat accordingly. One cause of anemia is deficiency of a nutrient  
necessary for erythropoiesis (process for producing red blood cells), such as, but not limited  
to, iron, B12, folate, etc. Another cause of anemia is blood loss, which can be acute or  
chronic. The blood loss can be caused by, for example, menorrhagia or bleeding from the  
20 gastrointestinal tract. Anemia is also frequently found in many chronic disorders, also called  
the anemia of chronic disease, which can be found in cancer and inflammatory bowel  
diseases.

**[0004]** Accordingly, it is desirable to provide new techniques for screening subjects for a  
hematological disorder, for determining a cause of a hematological disorder, for monitoring a  
25 subject with a hematological disorder, and/or determining a proper treatment of a subject with  
a hematological disorder.

## **BRIEF SUMMARY**

**[0004a]** In one aspect, the present invention provides a method of analyzing a blood sample  
of a mammal, the method comprising obtaining a cell-free mixture of the blood sample, the

cell-free mixture including cell-free DNA from a plurality of cell lineages; contacting DNA fragments in the cell-free mixture with an assay corresponding to one or more differentially-methylated regions, each of the one or more differentially-methylated regions specific to a particular hematological cell lineage by being hypomethylated or hypermethylated relative to other cell lineages; detecting a first number of methylated or unmethylated DNA fragments in the cell-free mixture at the one or more differentially-methylated regions based on signals obtained from the assay; determining a methylation level using the first number; and comparing the methylation level to one or more cutoff values as part of determining a classification of a hematological disorder in the mammal.

**[0004b]** In another aspect, the present invention provides a method of measuring an amount of cells of a particular cell lineage in a biological sample, the method comprising: obtaining a cell-free mixture of the biological sample, the cell-free mixture including cell-free DNA from a plurality of cell lineages; contacting DNA fragments in the cell-free mixture with an assay corresponding to one or more differentially-methylated regions, each of the one or more differentially-methylated regions specific to a particular cell lineage by being hypomethylated or hypermethylated relative to other cell lineages; detecting a first number of methylated or unmethylated DNA fragments in the cell-free mixture at the one or more differentially-methylated regions based on signals obtained from the assay; determining a first methylation level using the first number; obtaining one or more calibration data points, wherein each calibration data point specifies (1) an amount of cells of the particular cell lineage and (2) a calibration methylation level, and wherein the one or more calibration data points are determined from a plurality of calibration samples; comparing the first methylation level to a calibration methylation level of at least one calibration data point; and estimating the amount of cells of the particular cell lineage in the biological sample based on the comparing.

**[0004c]** In yet another aspect, the present invention provides for a computer product comprising a computer readable medium storing a plurality of instructions for controlling a system to perform a method of the present disclosure.

**[0004d]** In yet another aspect, the present invention provides for a system comprising: the computer product of the present disclosure; and one or more processors for executing instructions stored on the computer readable medium.

**[0004e]** In yet another aspect, the present invention provides for a system configured to perform a method of the present disclosure.

**[0004f]** In yet another aspect, the present invention provides for a computer product comprising a computer readable medium storing a plurality of instructions for controlling a

system to analyze a blood sample of a mammal by performing: detecting a first number of methylated or unmethylated DNA fragments in a cell-free mixture of the blood sample at one or more differentially-methylated regions based on signals obtained from an assay, each of the one or more differentially-methylated regions specific to a particular hematological cell lineage by being hypomethylated or hypermethylated relative to other cell lineages; determining a methylation level using the first number; and comparing the methylation level to one or more cutoff values as part of determining a classification of a hematological disorder in the mammal.

**[0004g]** In yet another aspect, the present invention provides for a computer product

comprising a computer readable medium storing a plurality of instructions for controlling a system to measure an amount of cells of a particular cell lineage in a biological sample by performing: detecting a first number of methylated or unmethylated DNA fragments in a cell-free mixture of the biological sample at one or more differentially-methylated regions based on signals obtained from an assay, each of the one or more differentially-methylated regions specific to a particular cell lineage by being hypomethylated or hypermethylated relative to other cell lineages; determining a first methylation level using the first number; obtaining one or more calibration data points, wherein each calibration data point specifies (1) an amount of cells of the particular cell lineage and (2) a calibration methylation level, and wherein the one or more calibration data points are determined from a plurality of calibration samples; comparing the first methylation level to a calibration methylation level of at least one calibration data point; and estimating the amount of cells of the particular cell lineage in the biological sample based on the comparing.

**[0005]** Some examples provide systems, methods, and apparatuses for detecting hematological disorders using cell-free DNA in a blood sample, e.g., using plasma or serum.

For example, an assay can target one or more differentially-methylated regions specific to a particular hematological cell lineage (e.g., erythroblasts). A methylation level can be quantified from the assay to determine an amount of methylated or unmethylated DNA fragments in a cell-free mixture of the blood sample. The methylation level can be compared  
5 to one or more cutoff values, e.g., that correspond to a normal range for the particular hematological cell lineage as part of determining a level of a hematological disorder. Some examples can measure an amount of DNA from the particular hematological cell lineage (e.g., erythroblast DNA) in a blood sample in a similar manner using one or more methylation levels.

10 **[0006]** Such an analysis can provide a detection of a hematological disorder without performing the invasive procedure of a bone marrow biopsy. For example, our results demonstrate that bone marrow cells contribute a significant proportion to the circulating cell-free DNA. An analysis of the methylation signatures of the hematopoietic cells in the circulating cell-free DNA can reflect the status of the bone marrow cells. Such examples can  
15 be particularly useful for the monitoring of response of the bone marrow to treatments, for example, the response to oral iron therapy in patients with iron deficiency anemia. Examples can also be used for assigning patients for different procedures, e.g., a bone marrow biopsy or less invasive investigations.

**[0007]** Other examples are directed to systems and computer readable media associated  
20 with methods described herein.

**[0008]** A better understanding of the nature and advantages of embodiments of the present invention may be gained with reference to the following detailed description and the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

25 **[0009]** FIG. 1 shows methylation densities of the CpG sites within the promoter of the ferrochelatase (FECH) gene according to embodiments of the present invention.

**[0010]** FIGS. 2A and 2B show an analysis of universally methylated and unmethylated DNA using the digital PCR assays designed for detecting methylated and unmethylated DNA according to embodiments of the present invention.

30 **[0011]** FIG. 3A is a plot showing a correlation between E% in the blood cells and the number of nucleated RBC (erythroblasts) according to embodiments of the present invention.

5 after treatment, repeating the assay to determine an updated methylation level;  
6 and  
7 determining whether to continue to perform the treatment based on the updated  
8 methylation level.

1 9. The method of claim 8, wherein determining whether to continue to  
2 perform the treatment includes:  
3 stopping the treatment, increasing a dose of the treatment, or pursuing a different  
4 treatment when the updated methylation level has not changed relative to the methylation level to  
5 within a specified threshold.

1 10. The method of claim 8, wherein determining whether to continue to  
2 perform the treatment includes:  
3 continuing the treatment when the updated methylation level has changed relative  
4 to the methylation level to within a specified threshold.

1 11. The method of claim 1, further comprising:  
2 determining that the hematological disorder exists based on comparing the  
3 methylation level to one or more cutoff values; and  
4 performing a bone marrow biopsy in response to determining that the  
5 hematological disorder exists.

1 12. The method of claim 1, wherein the assay is a PCR assay or a sequencing  
2 assay.

1 13. The method of claim 1, wherein the one or more differentially-methylated  
2 regions comprise CpG sites.

1 14. The method of claim 13, wherein a first region of the one or more  
2 differentially-methylated region comprises a plurality of CpG sites that are within 100 bp of each  
3 other, and wherein the plurality of CpG sites are all hypomethylated or hypermethylated.

1 15. The method of claim 14, wherein the plurality of CpG sites span 100 bp or  
2 less on a reference genome corresponding to the mammal.

1                   27.     The method of claim 1, wherein the cell-free mixture is plasma.

1                   28.     A method of measuring an amount of cells of a particular cell lineage in a  
2 biological sample, the method comprising:

3                   obtaining a cell-free mixture of the biological sample, the cell-free mixture  
4 including cell-free DNA from a plurality of cell lineages;

5                   contacting DNA fragments in the cell-free mixture with an assay corresponding to  
6 one or more differentially-methylated regions, each of the one or more differentially-methylated  
7 regions specific to a particular cell lineage by being hypomethylated or hypermethylated relative  
8 to other cell lineages;

9                   detecting a first number of methylated or unmethylated DNA fragments in the  
10 cell-free mixture at the one or more differentially-methylated regions based on signals obtained  
11 from the assay;

12                  determining a first methylation level using the first number;

13                  obtaining one or more calibration data points, wherein each calibration data point  
14 specifies (1) the amount of cells of the particular cell lineage and (2) a calibration methylation  
15 level, and wherein the one or more calibration data points are determined from a plurality of  
16 calibration samples;

17                  comparing the first methylation level to a calibration methylation level of at least  
18 one calibration data point; and

19                  estimating the amount of cells of the particular cell lineage in the biological  
20 sample based on the comparing.

1                   29.     The method of claim 28, wherein the particular cell lineage is a particular  
2 hematological cell lineage.

1                   30.     The method of claim 28, wherein the one or more calibration data points  
2 are a plurality of calibration data points, and wherein the calibration data points form a  
3 calibration curve.

1                   31.     A computer product comprising a computer readable medium storing a  
2 plurality of instructions for controlling a system to perform the method of any one of claims 1-30.

1                   32.     A system comprising:  
2                   the computer product of claim 31; and  
3                   one or more processors for executing instructions stored on the computer readable  
4 medium.

1                   33.     A system configured to perform the method of any one of claims 1-30.

1                   34.     A computer product comprising a computer readable medium storing a  
2 plurality of instructions for controlling a system to analyze a blood sample of a mammal by  
3 performing:  
4                   detecting a first number of methylated or unmethylated DNA fragments in a cell-  
5 free mixture of the blood sample at one or more differentially-methylated regions based on  
6 signals obtained from an assay, each of the one or more differentially-methylated regions  
7 specific to a particular hematological cell lineage by being hypomethylated or hypermethylated  
8 relative to other cell lineages;  
9                   determining a methylation level using the first number; and  
10                  comparing the methylation level to one or more cutoff values as part of  
11 determining a classification of a hematological disorder in the mammal.

1                   35.     A computer product comprising a computer readable medium storing a  
2 plurality of instructions for controlling a system to measure an amount of cells of a particular cell  
3 lineage in a biological sample by performing:  
4                   detecting a first number of methylated or unmethylated DNA fragments in a cell-  
5 free mixture of the biological sample at one or more differentially-methylated regions based on  
6 signals obtained from an assay, each of the one or more differentially-methylated regions  
7 specific to a particular cell lineage by being hypomethylated or hypermethylated relative to other  
8 cell lineages;  
9                   determining a first methylation level using the first number;  
10                  obtaining one or more calibration data points, wherein each calibration data point  
11 specifies (1) an amount of cells of the particular cell lineage and (2) a calibration methylation  
12 level, and wherein the one or more calibration data points are determined from a plurality of  
13 calibration samples;