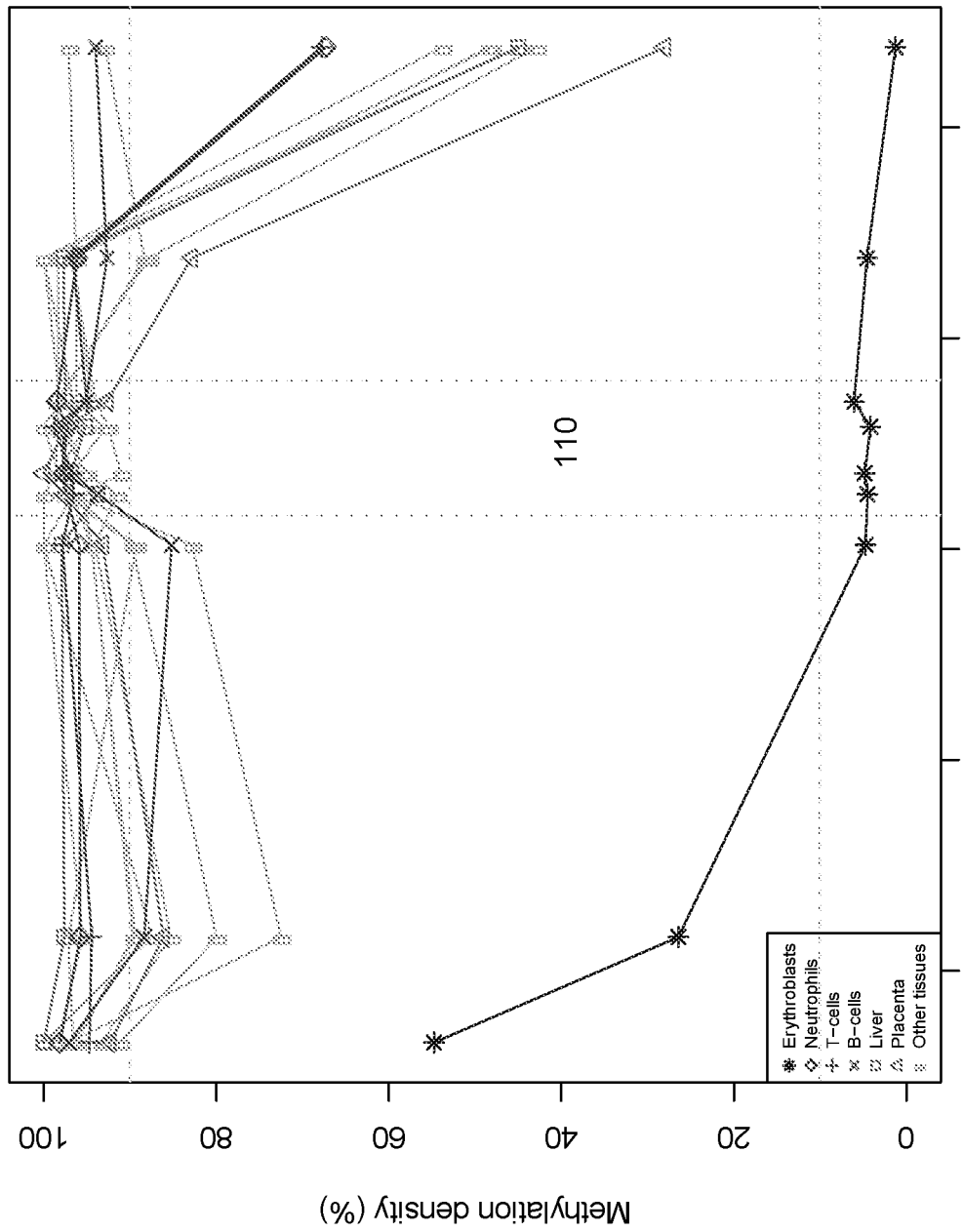
Methylation density (%)
0
20
40
60
80
100
110
55250450
55250500
55250550
55250600
55250650
Genomic coordinate (chr18)
Erythroblasts
Neutrophils
T−cells
B−cells
Liver
Placenta
Other tissues

FIG. 1

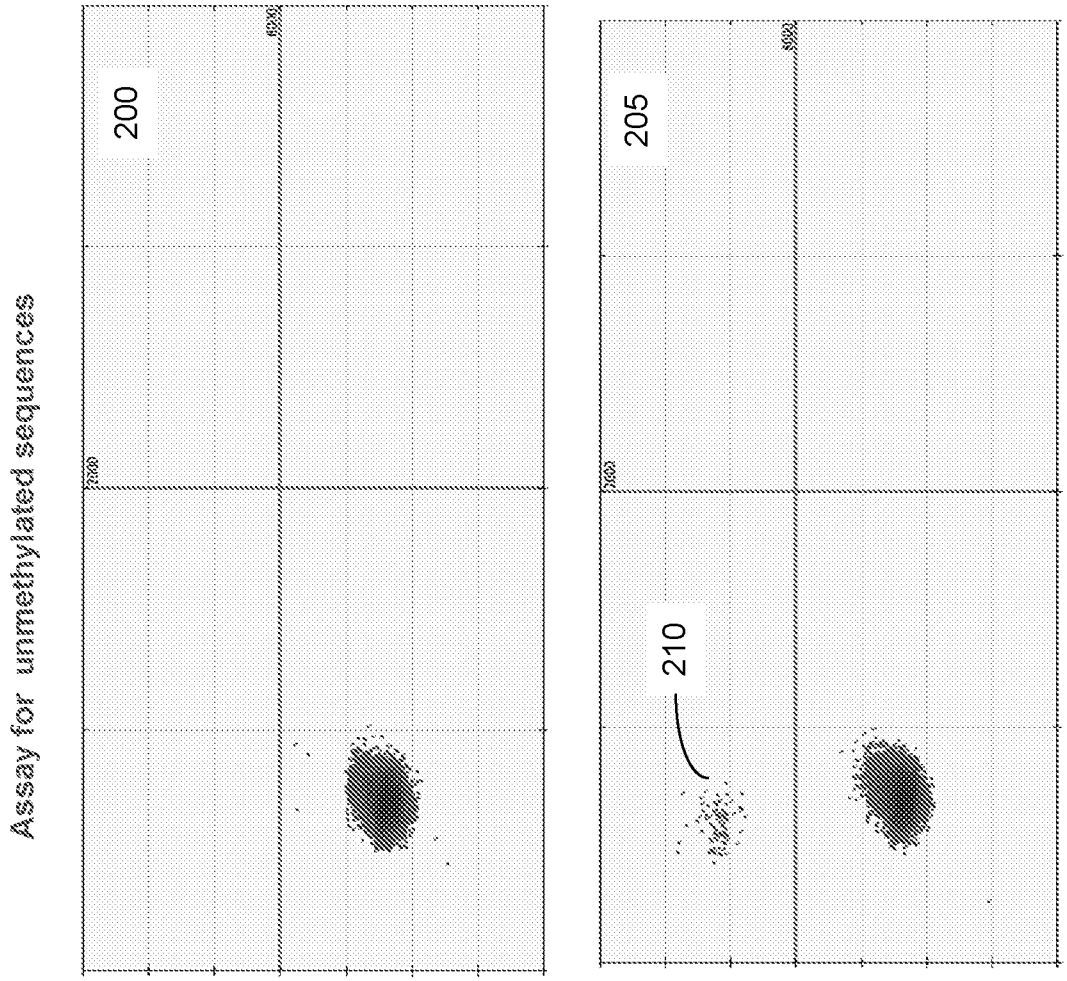
Assay for unmethylated sequences
200
205
210
Universally methylated DNA
Universal unmethylated DNA
Relative fluorescence signal for unmethylated sequences
Relative fluorescence signal for methylated sequences

FIG. 2A

Universally methylated DNA

Universal unmethylated DNA

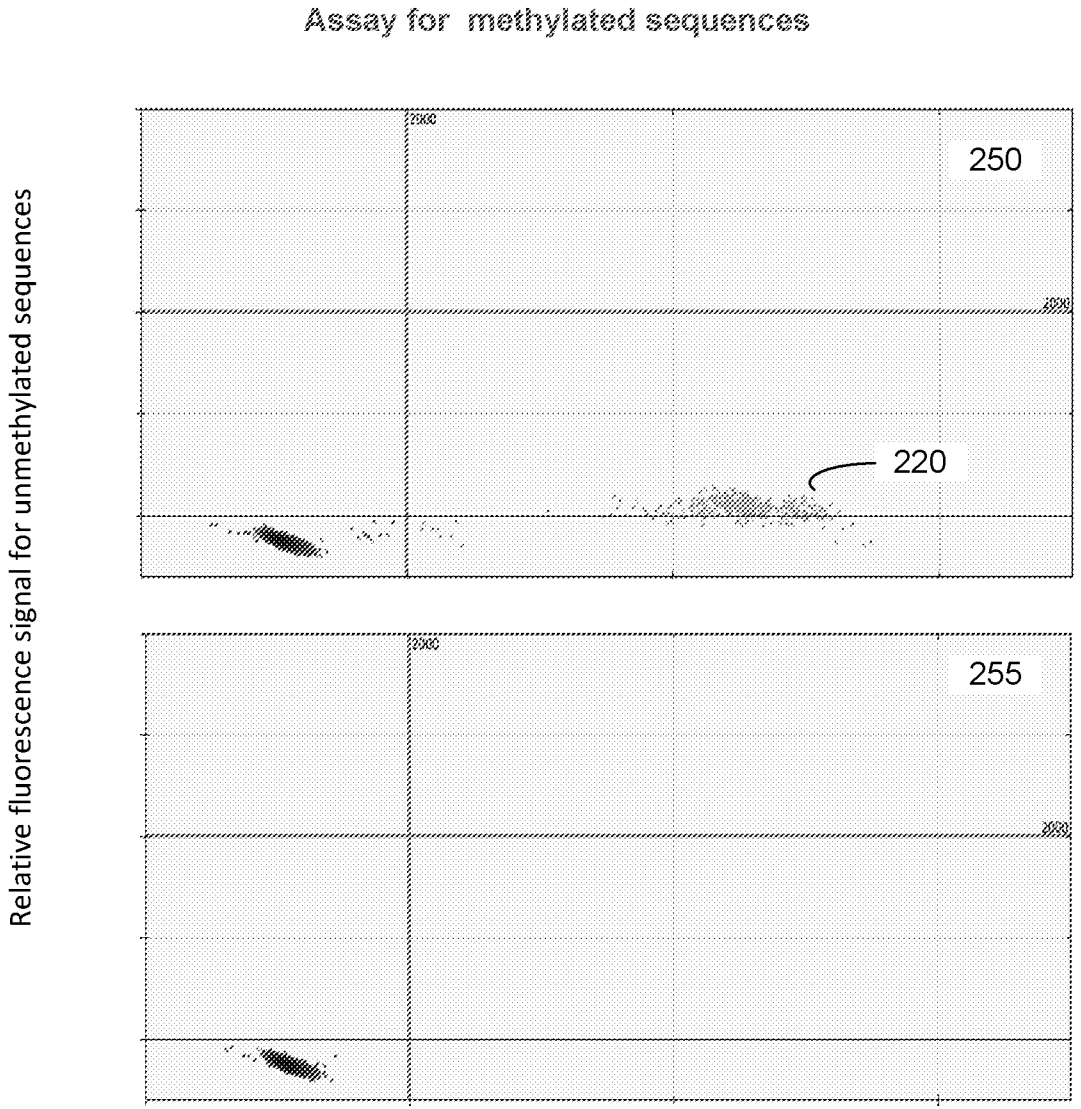

FIG. 2B

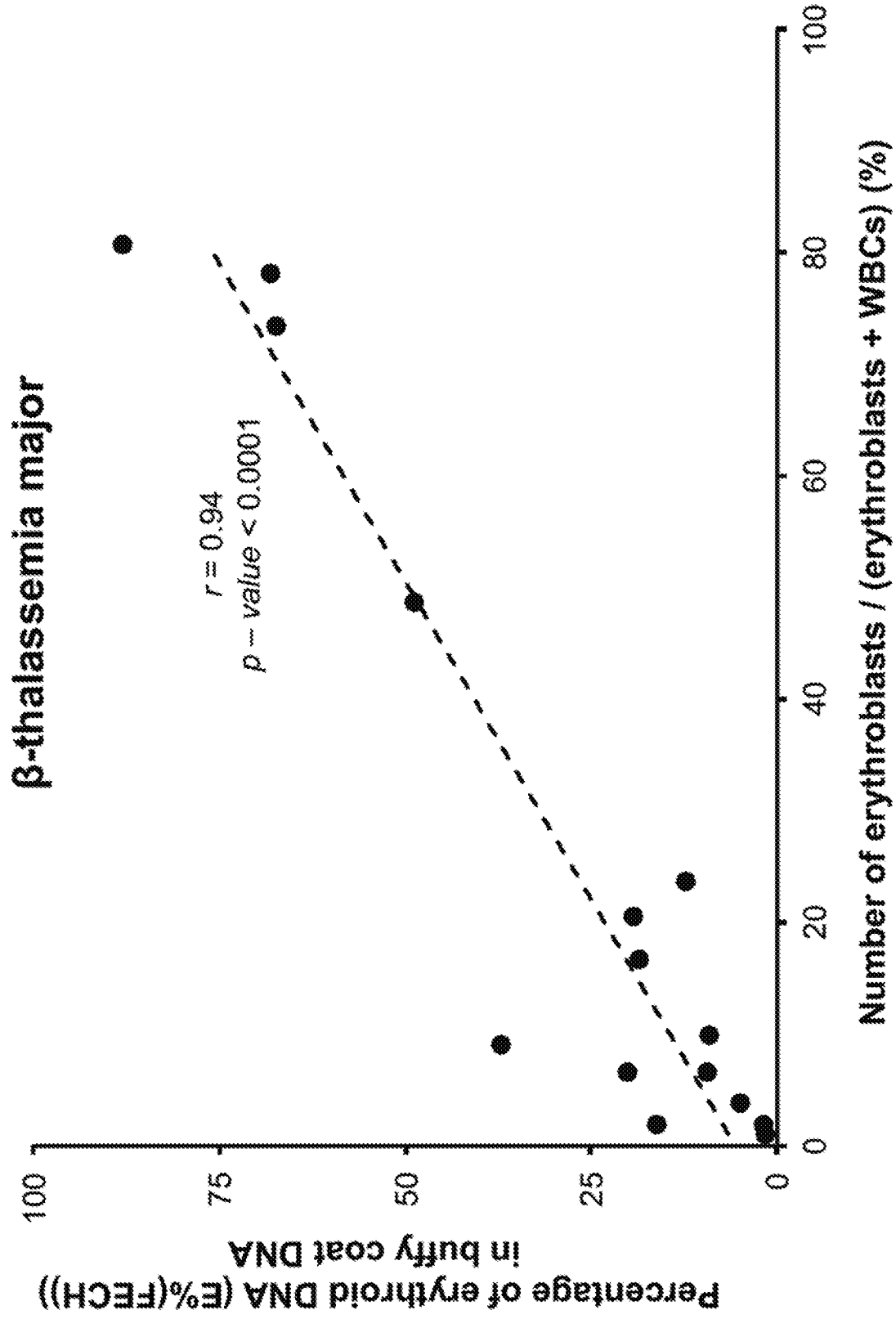
β-thalassemia major
r = 0.94
p – value < 0.0001
Percentage of erythroid DNA (E%(FECH))
in buffy coat DNA
Number of erythroblasts / (erythroblasts + WBCs) (%)
0
25
50
75
100
0
20
40
60
80
100

FIG. 3A

2600

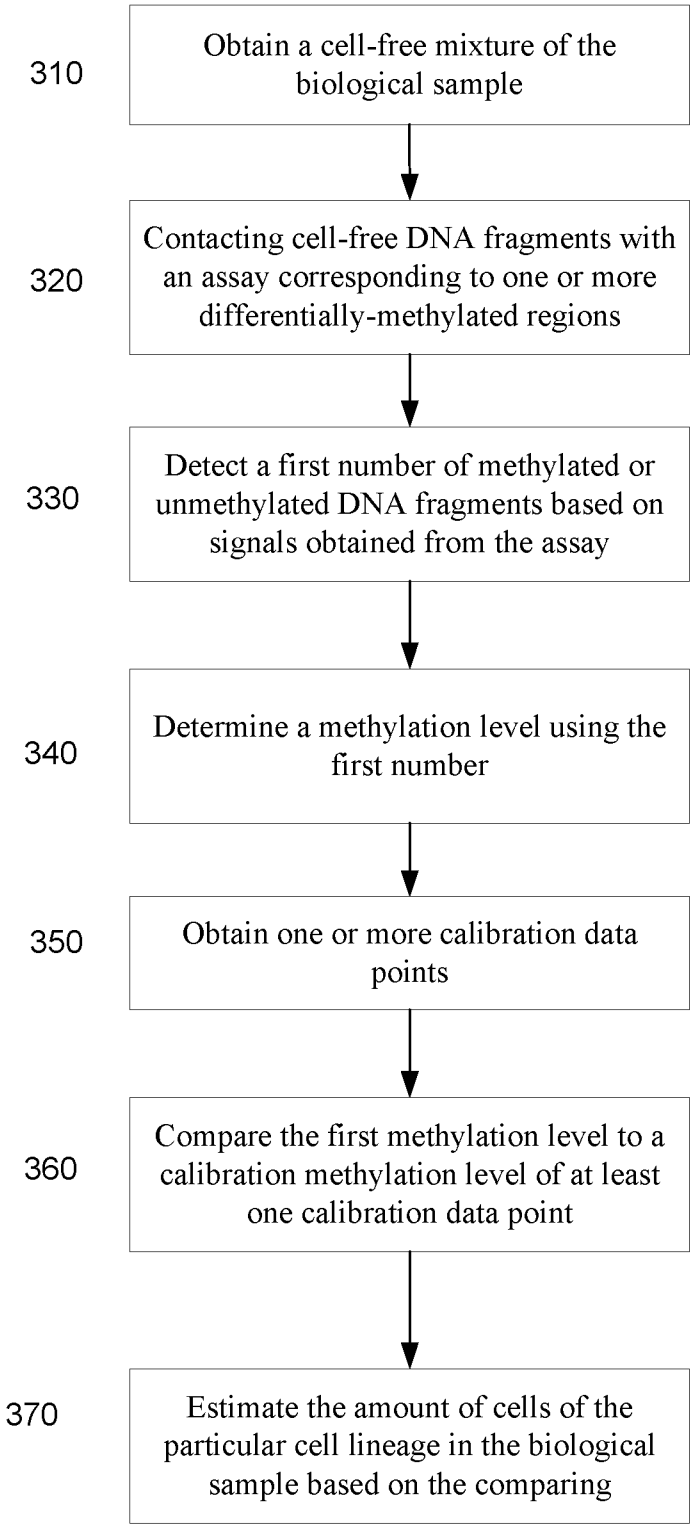
310
Obtain a cell-free mixture of the biological sample
320
Contacting cell-free DNA fragments with an assay corresponding to one or more differentially-methylated regions
330
Detect a first number of methylated or unmethylated DNA fragments based on signals obtained from the assay
340
Determine a methylation level using the first number
350
Obtain one or more calibration data points
360
Compare the first methylation level to a calibration methylation level of at least one calibration data point
370
Estimate the amount of cells of the particular cell lineage in the biological sample based on the comparing

FIG. 3B

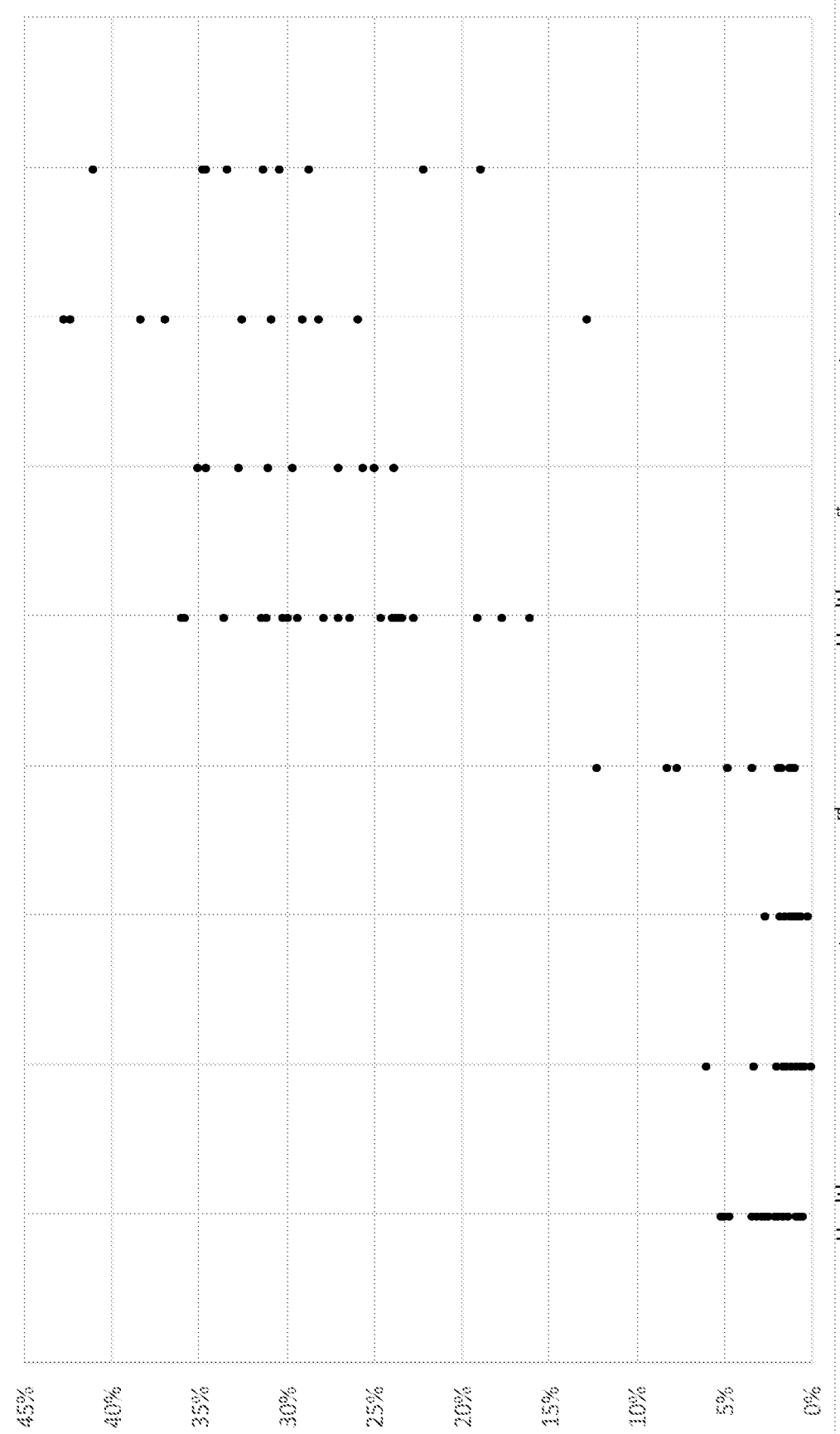
Unmeth%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Healthy non-pregnant subjects
1st trimester pregnant women
2nd trimester pregnant women
3rd trimester pregnant women
Healthy non-pregnant subjects
1st trimester pregnant women
2nd trimester pregnant women
3rd trimester pregnant women
Buffy coat
Plasma

FIG. 4

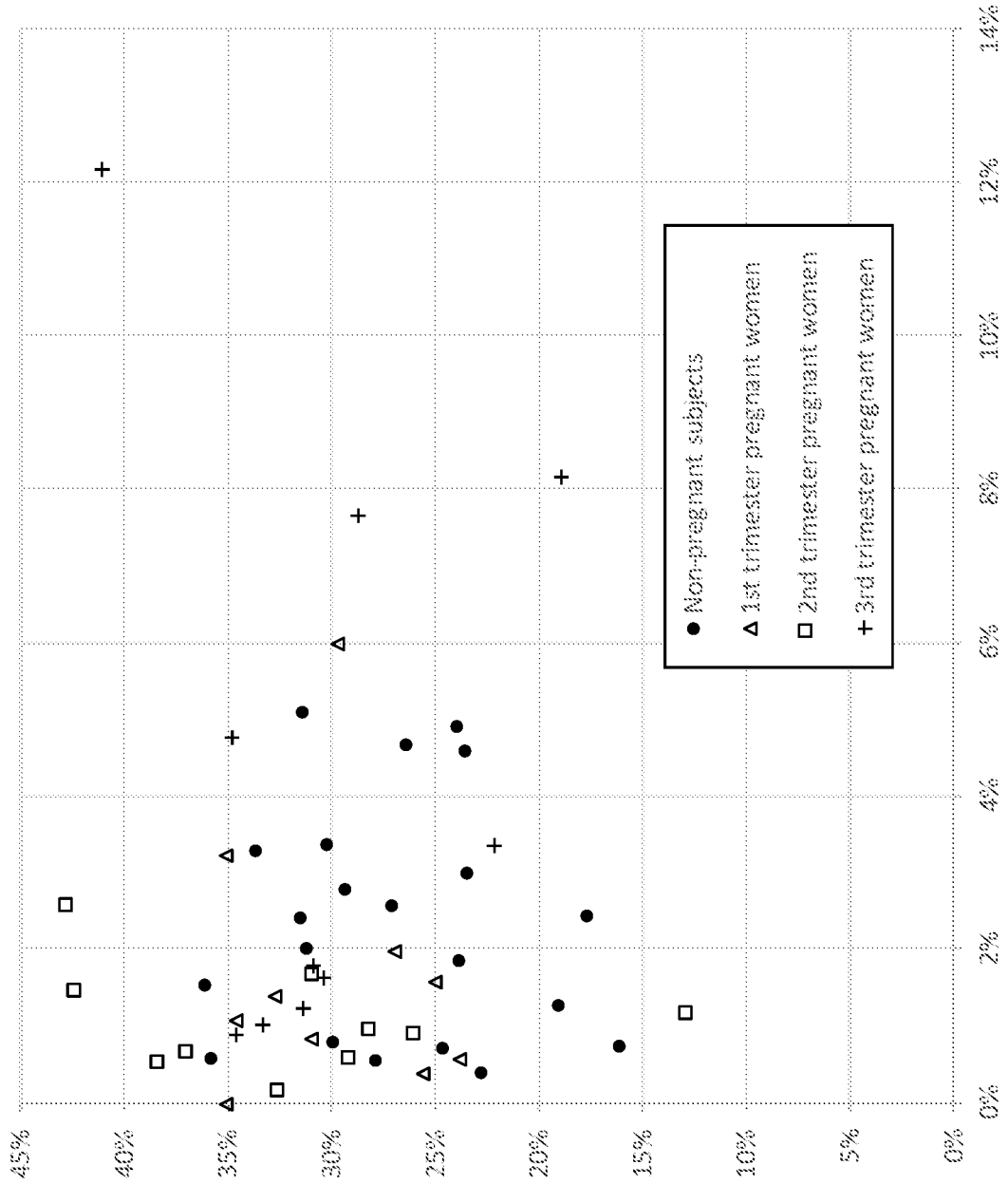
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
0%
2%
4%
6%
8%
10%
12%
14%
Unmeth% for plasma
Unmeth% for buffy coat
Non-pregnant subjects
1st trimester pregnant women
2nd trimester pregnant women
3rd trimester pregnant women

FIG. 5

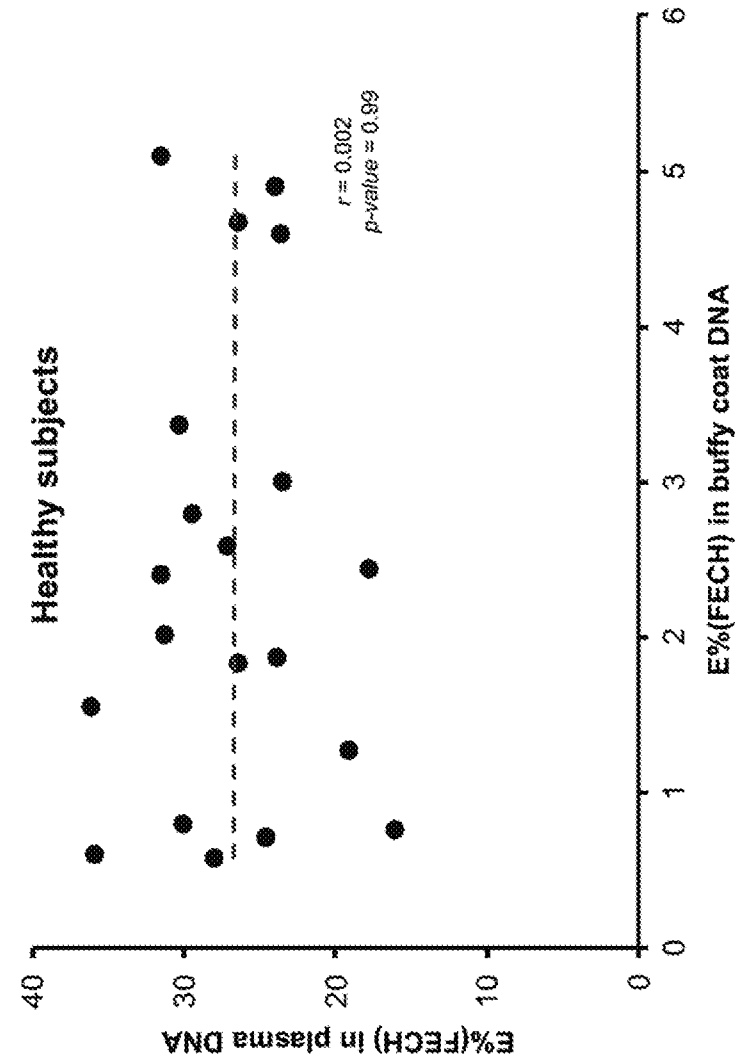
Healthy subjects
E%(FECH) in plasma DNA
E%(FECH) in buffy coat DNA
r = 0.002
p-value = 0.99
0
10
20
30
40
0
1
2
3
4
5
6

FIG. 6B

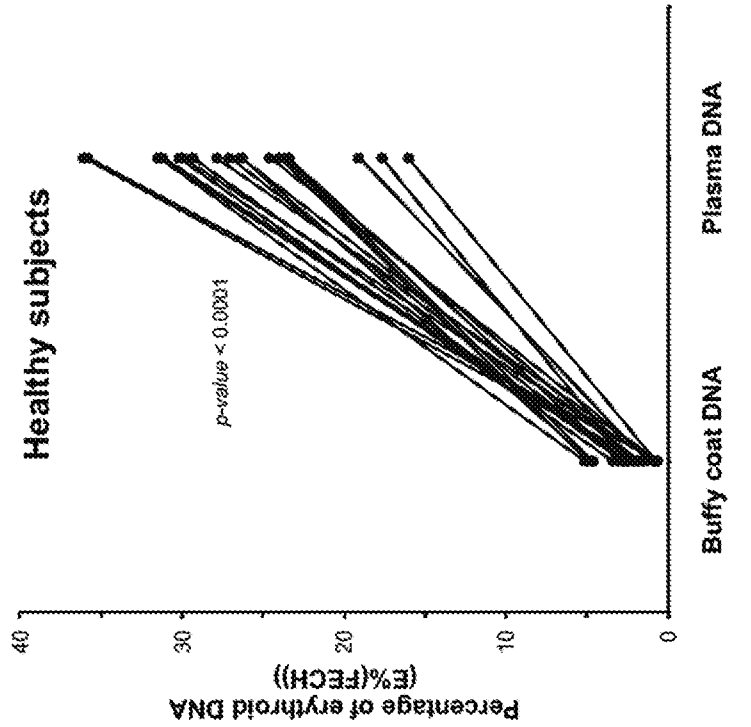
Healthy subjects
Percentage of erythroid DNA
(E%(FECH))
p-value < 0.0001
Buffy coat DNA
Plasma DNA
0
10
20
30
40

FIG. 6A

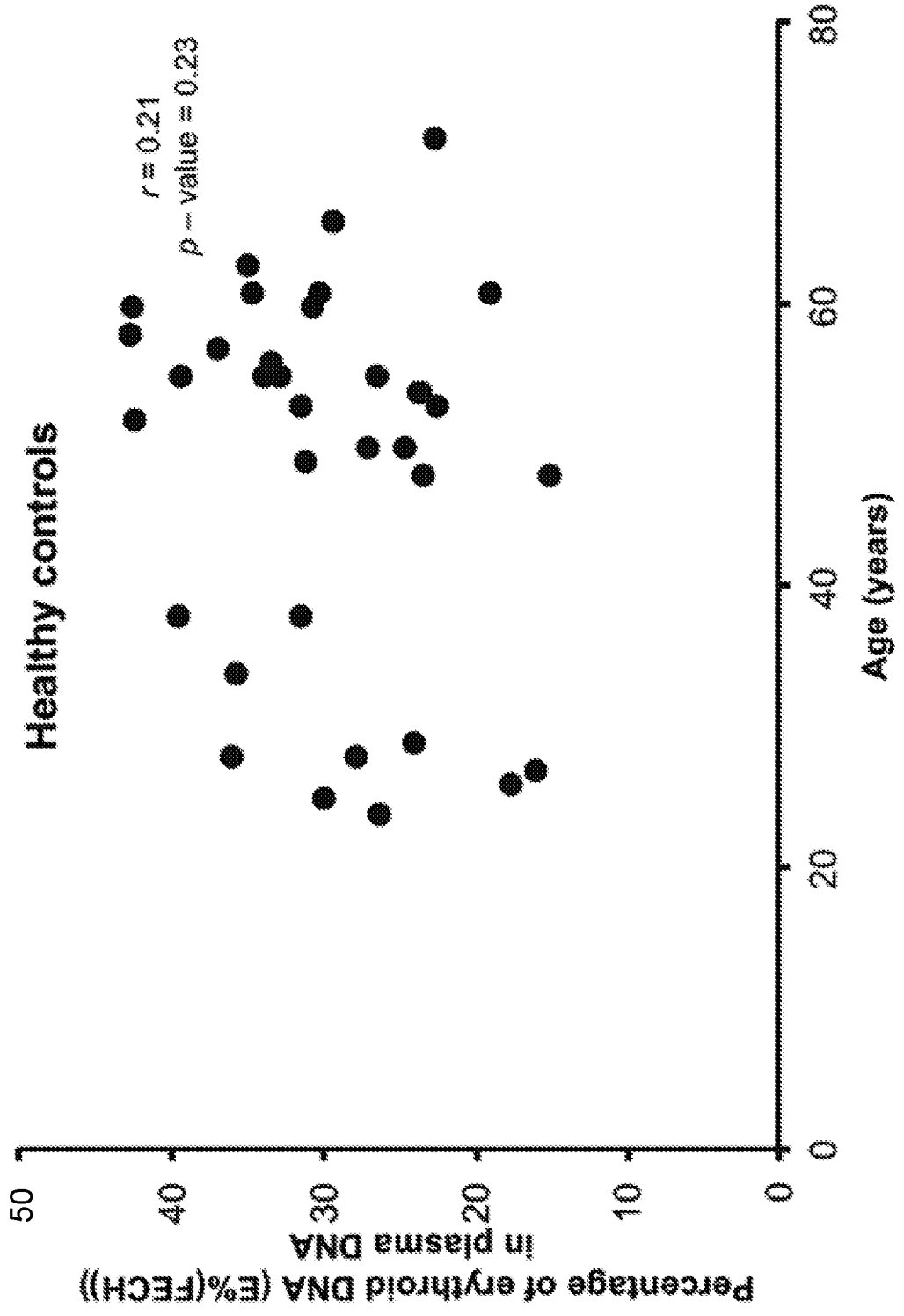
Healthy controls
r = 0.21
p – value = 0.23
Percentage of erythroid DNA (E%(FECH))
in plasma DNA
0
10
20
30
40
50
0
20
40
60
80
Age (years)

FIG. 7

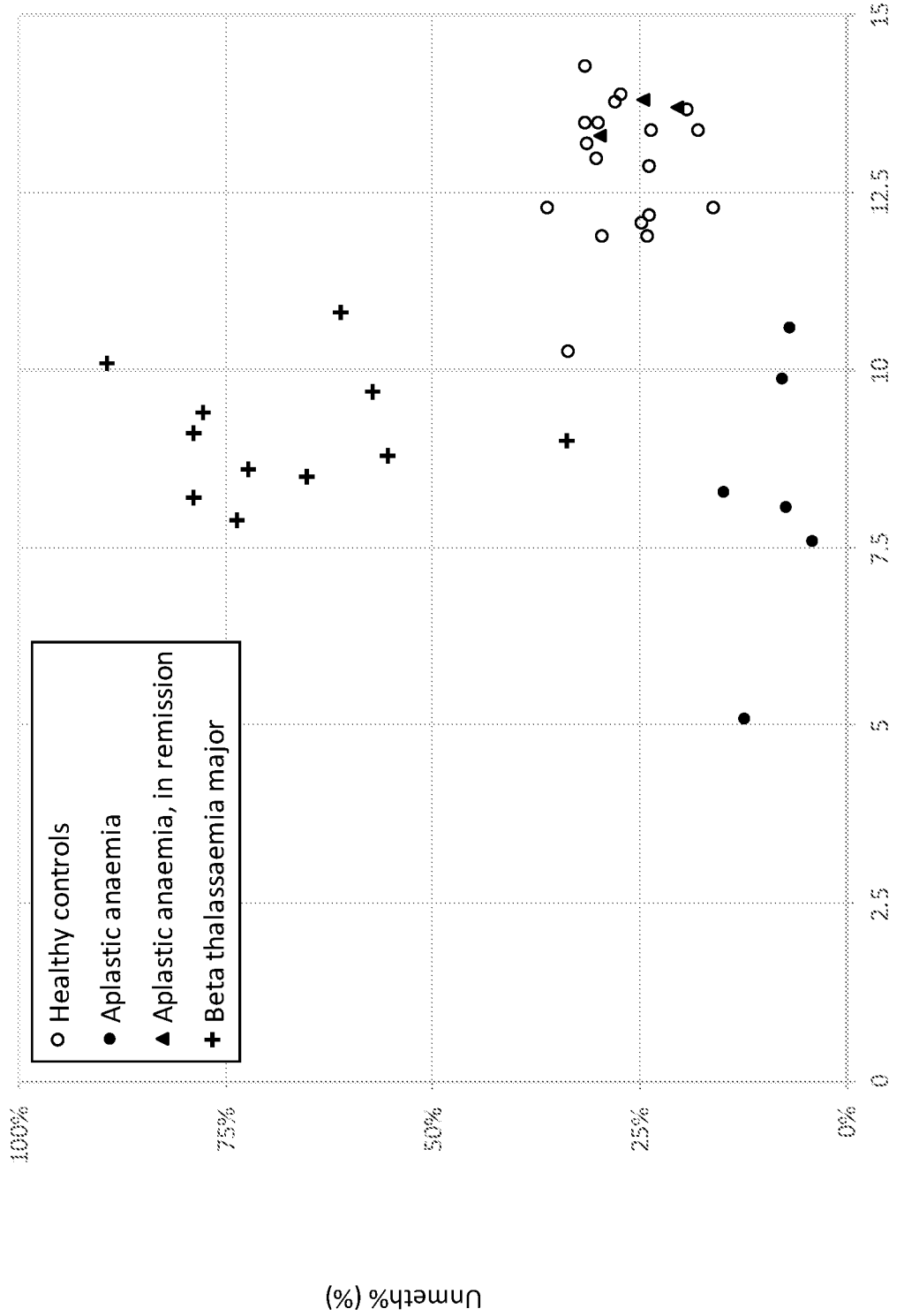
Healthy controls
Aplastic anaemia
Aplastic anaemia, in remission
Beta thalassaemia major
100%
75%
50%
25%
0%
Unmeth% (%)
0
2.5
5
7.5
10
12.5
15
Hemoglobin concentration (g/dL)

FIG. 8

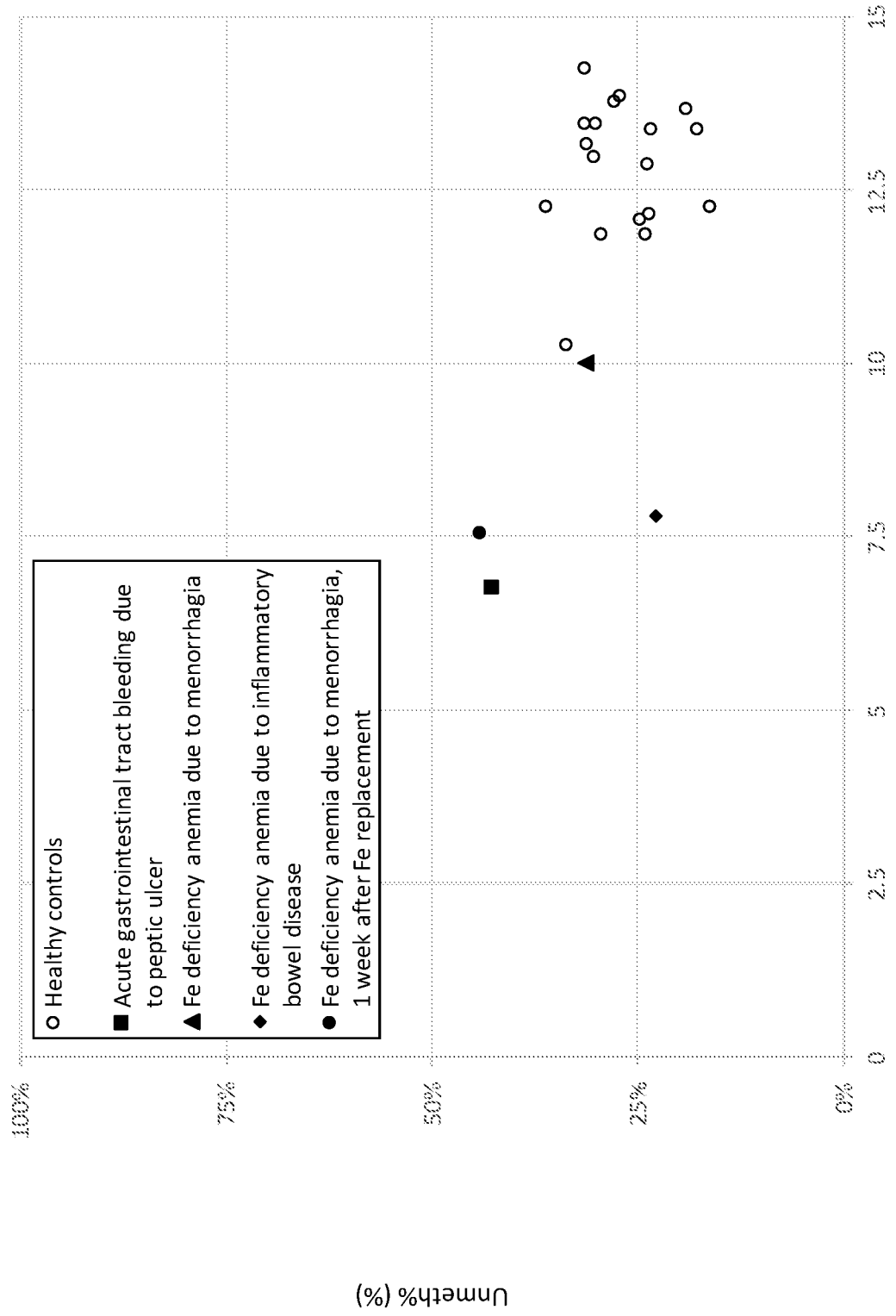
Healthy controls
Acute gastrointestinal tract bleeding due to peptic ulcer
Fe deficiency anemia due to menorrhagia
Fe deficiency anemia due to inflammatory bowel disease
Fe deficiency anemia due to menorrhagia, 1 week after Fe replacement
100%
75%
50%
25%
0%
Unmeth% (%)
0
2.5
5
7.5
10
12.5
15
Hemoglobin concentration (g/dL)

FIG. 9

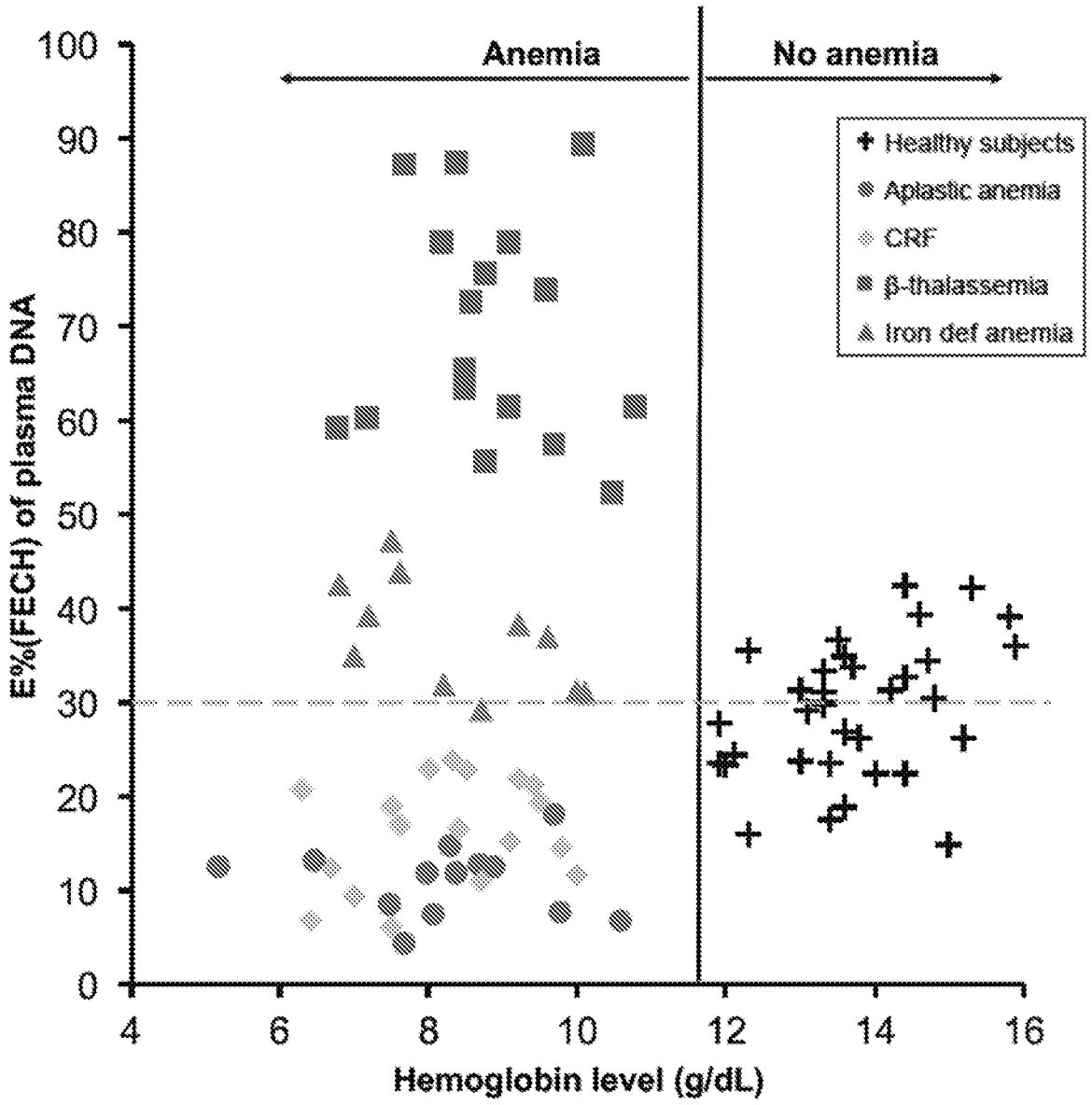
Anemia
No anemia
Healthy subjects
Aplastic anemia
CRF
β-thalassemia
Iron def anemia
E%(FECH) of plasma DNA
Hemoglobin level (g/dL)
0
10
20
30
40
50
60
70
80
90
100
4
6
8
10
12
14
16

FIG. 10

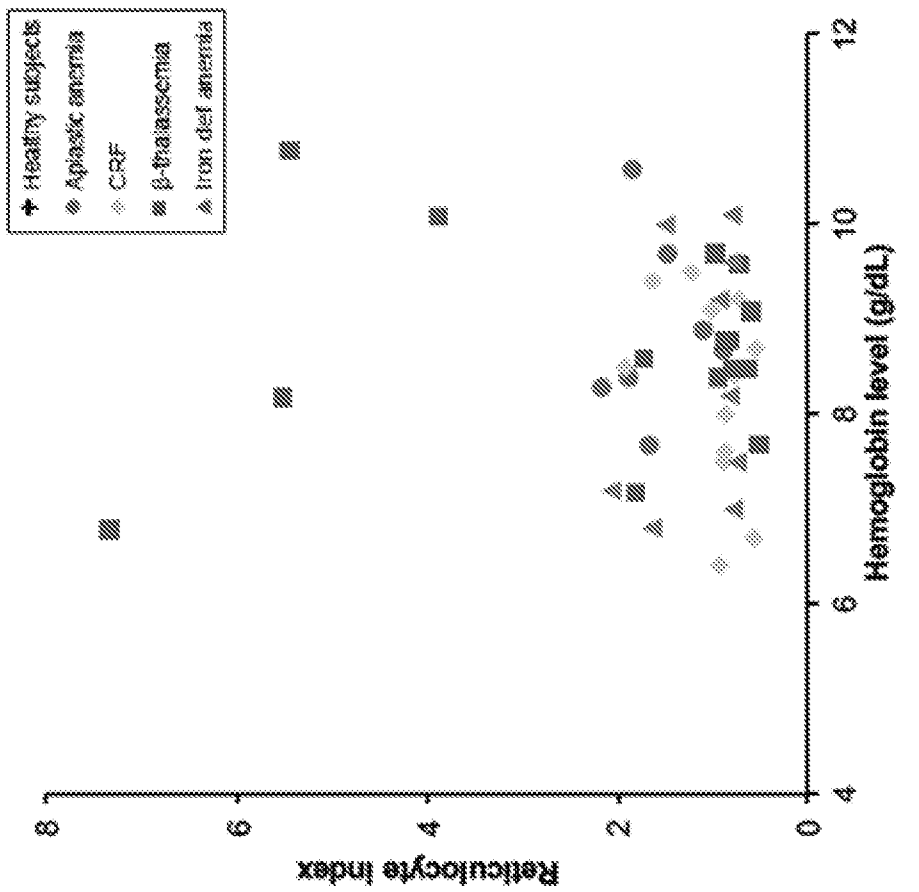

FIG. 11B

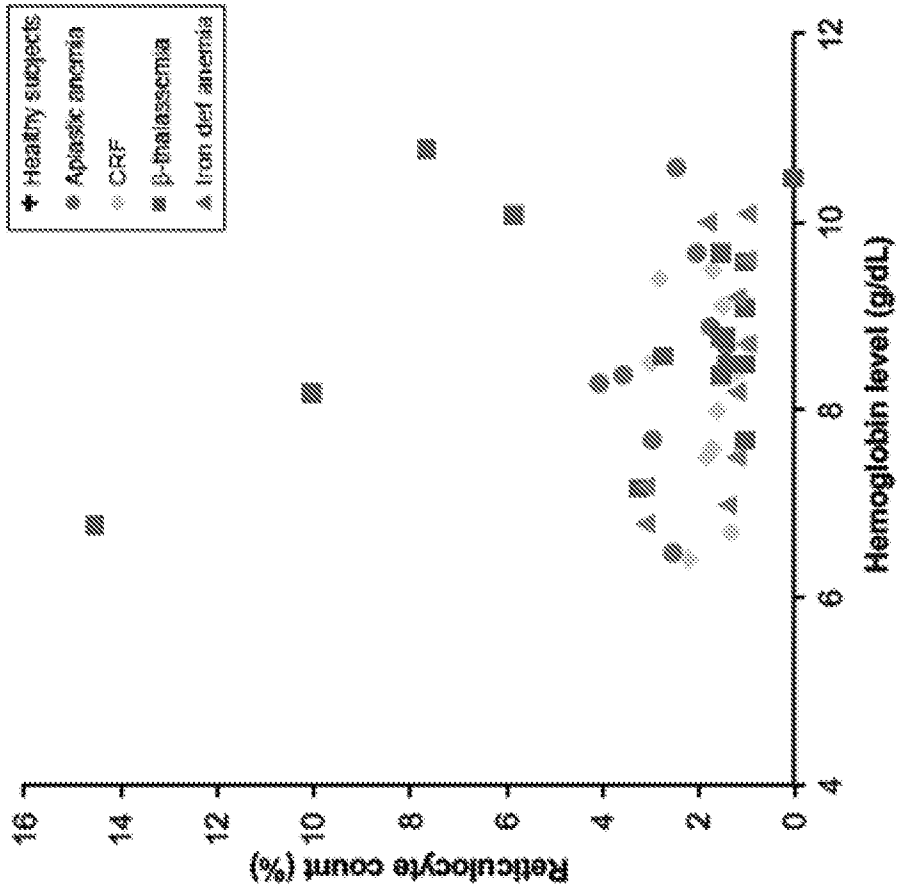

FIG. 11A

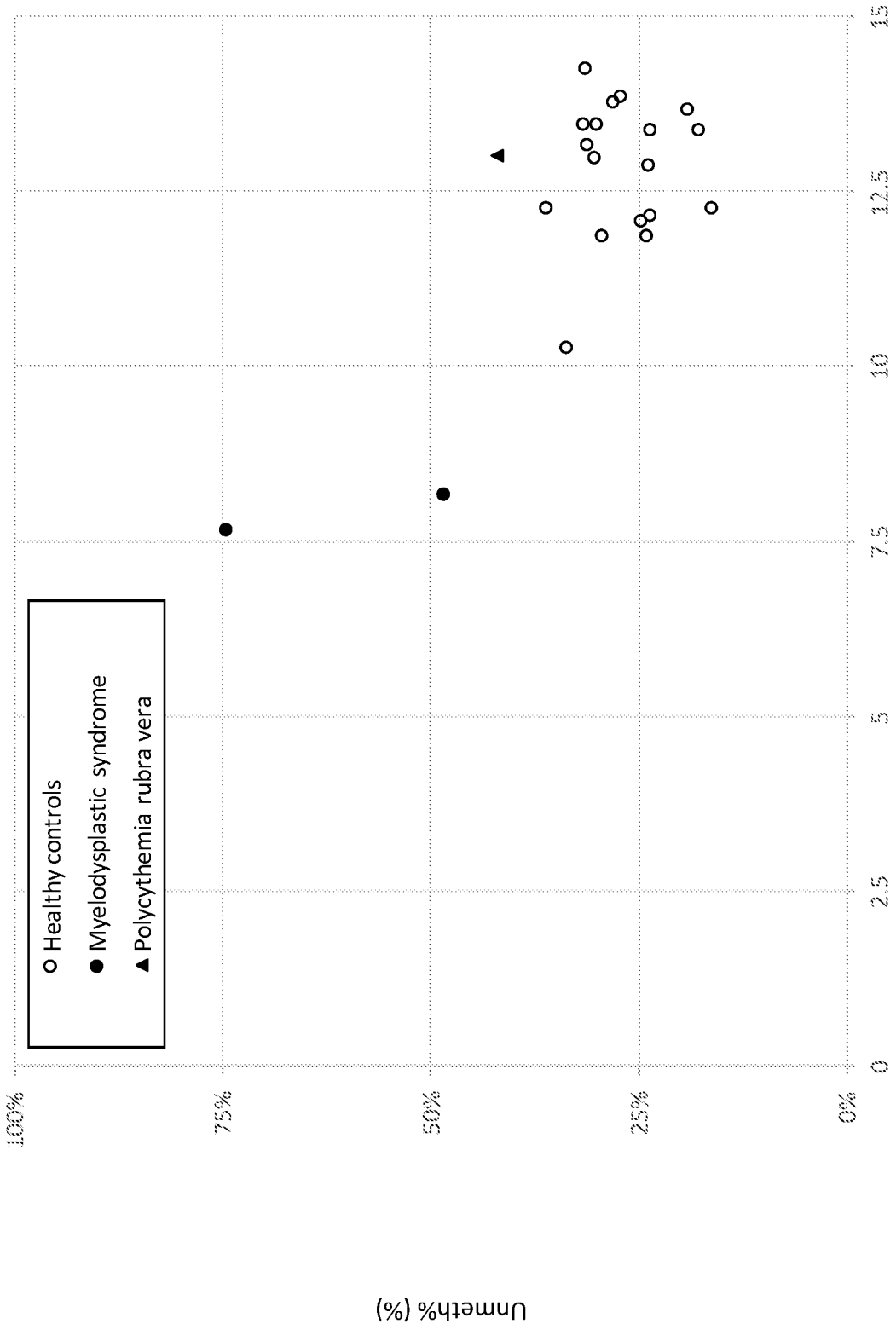
Healthy controls
Myelodysplastic syndrome
Polycythemia rubra vera
100%
75%
50%
25%
0%
Unmeth% (%)
0
2.5
5
7.5
10
12.5
15
Hemoglobin concentration (g/dL)

FIG. 12

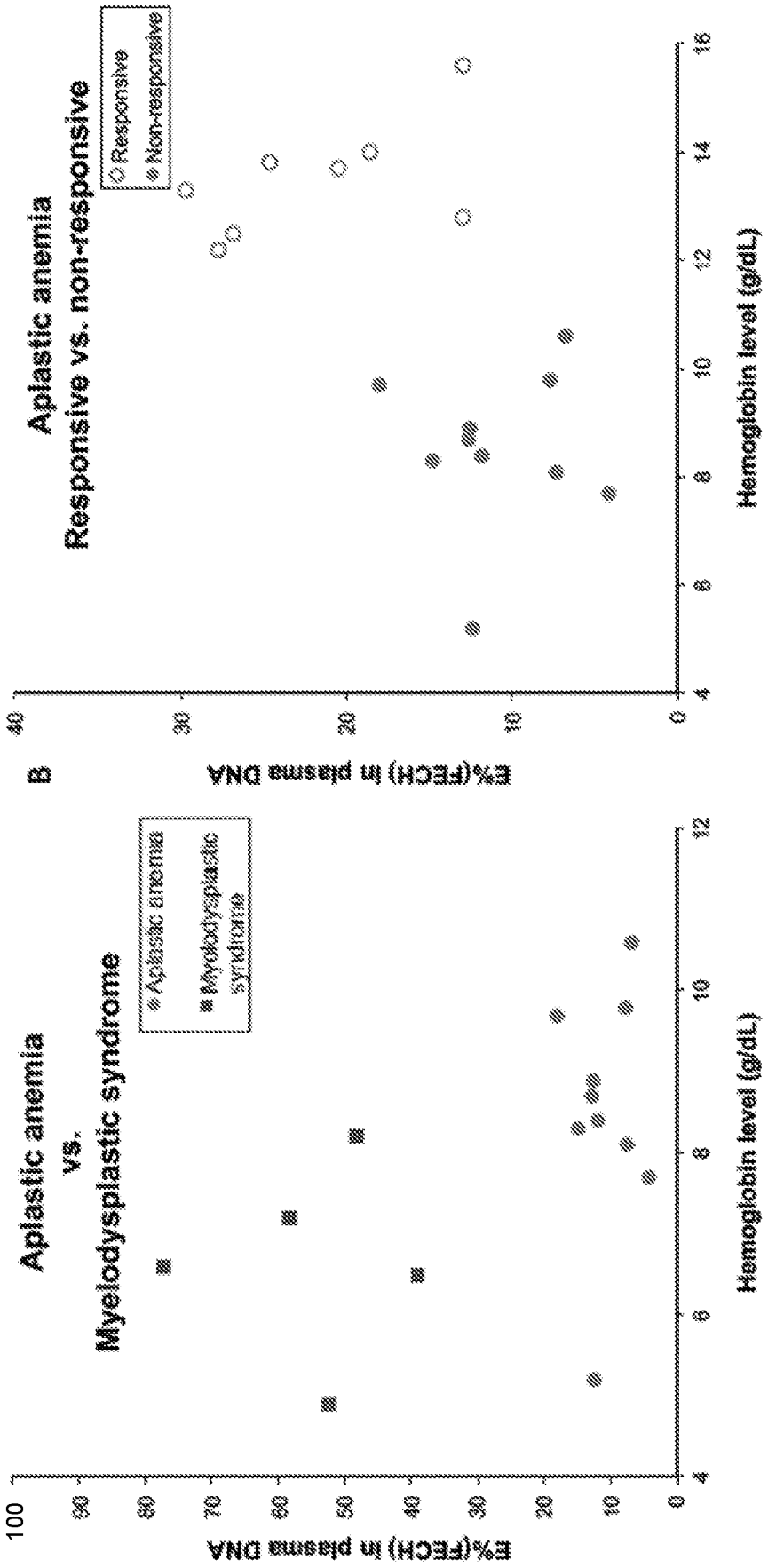

FIG. 13A

FIG. 13B

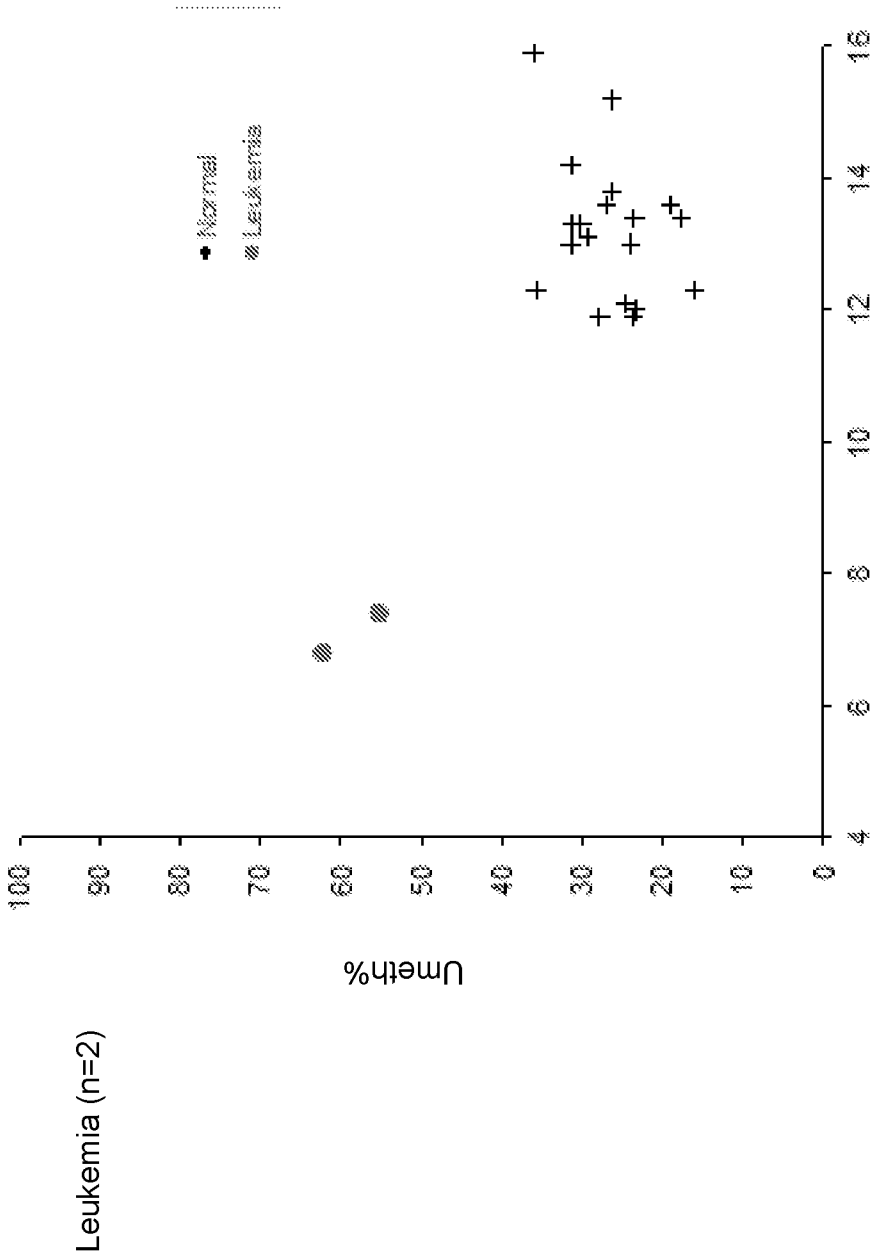
Leukemia (n=2)
Meth%
Hemoglobin level (g/dL)
Normal
Leukemia
100
90
80
70
60
50
40
30
20
10
0
4
6
8
10
12
14
16

FIG. 14

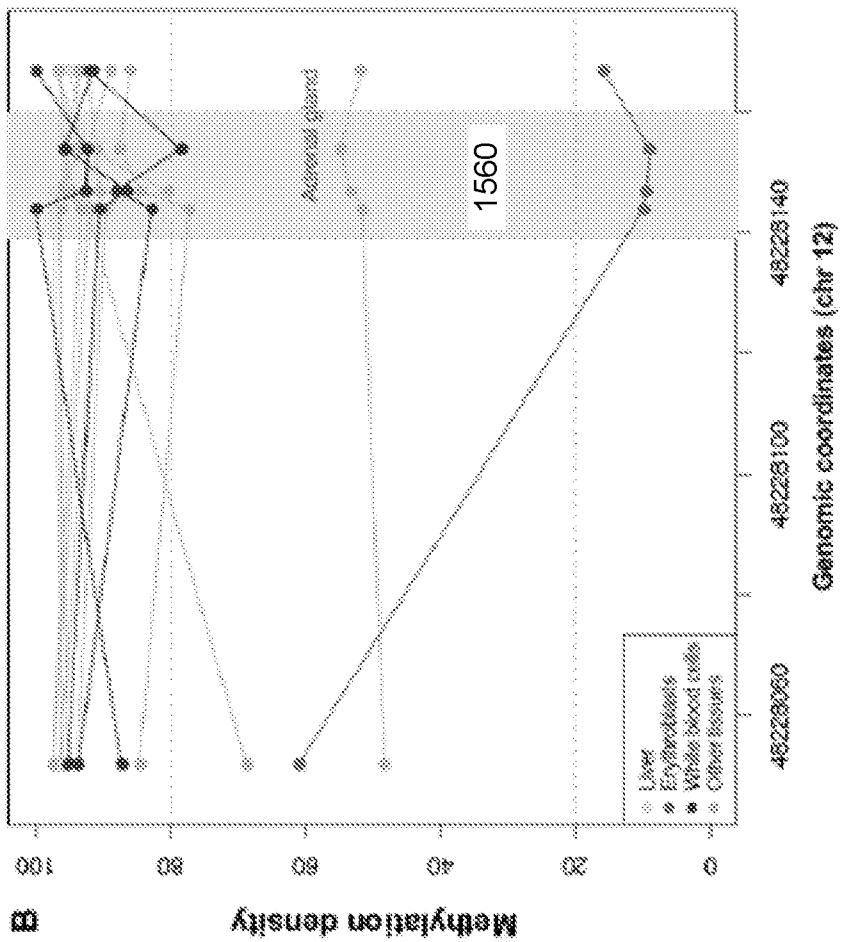

FIG. 15B

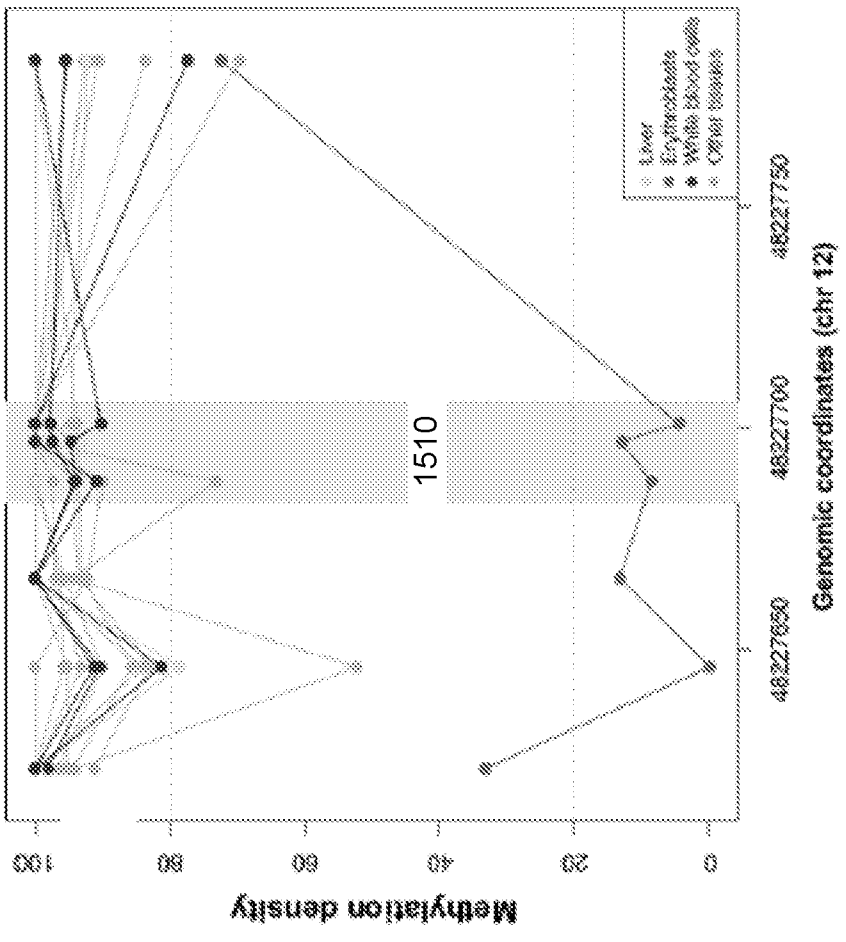

FIG. 15A

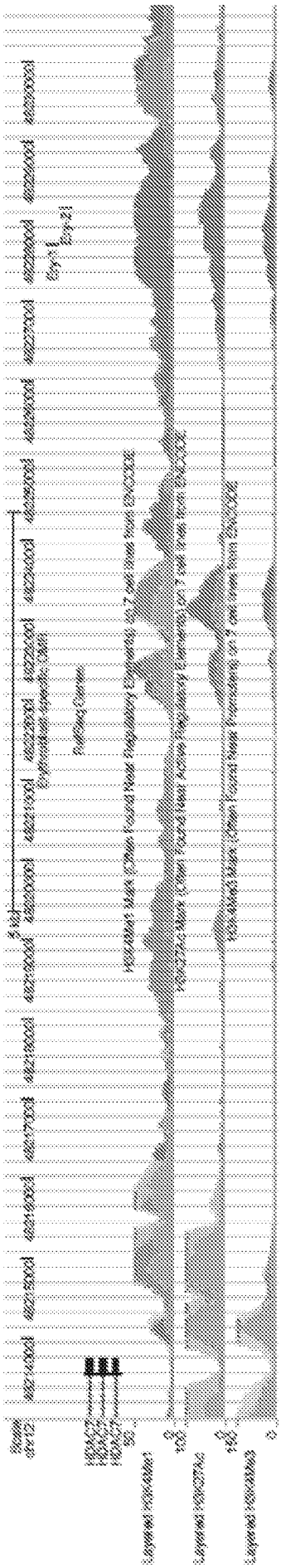

FIG. 16

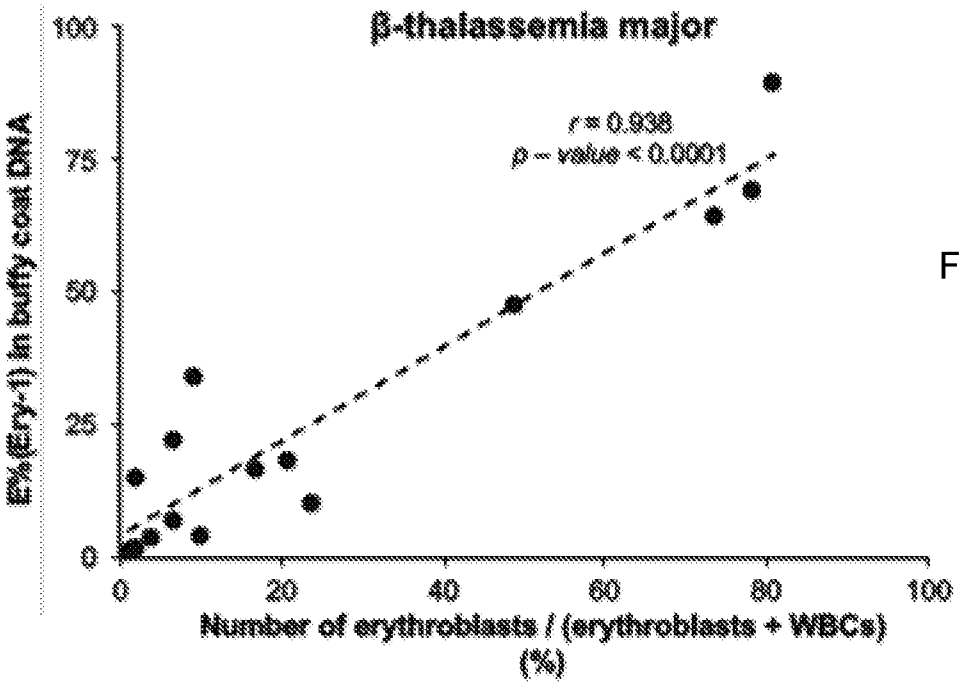

FIG. 17A

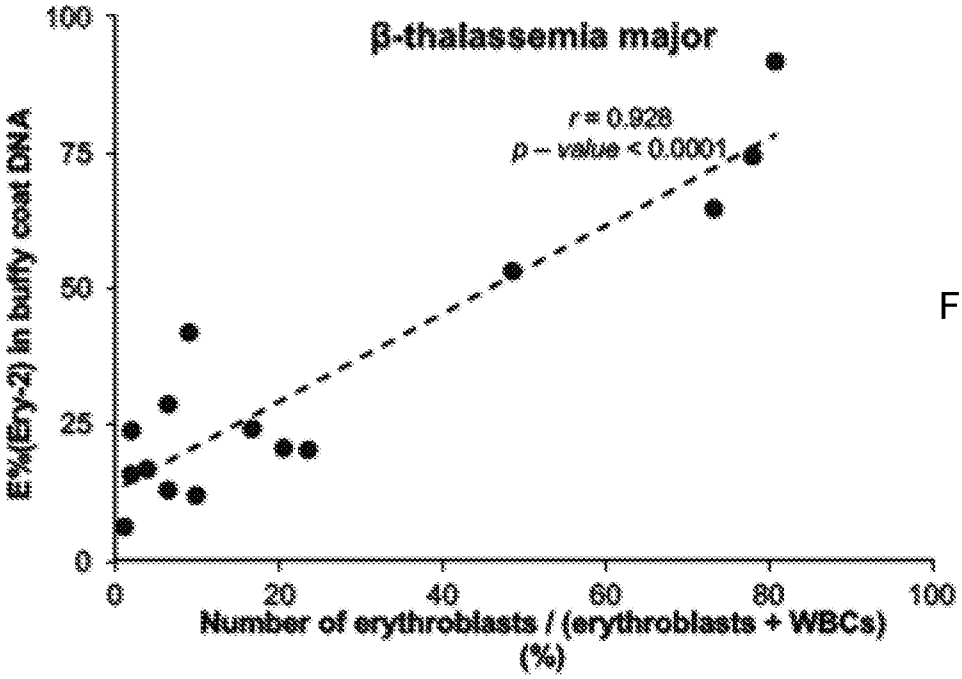

FIG. 17B

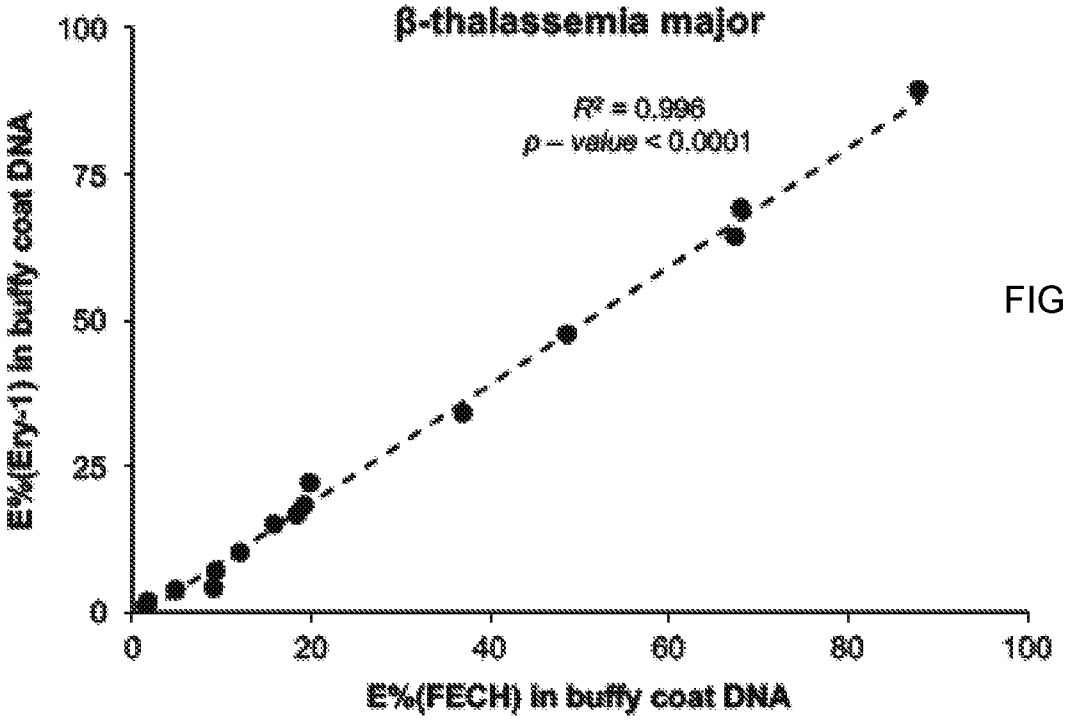

FIG. 18A

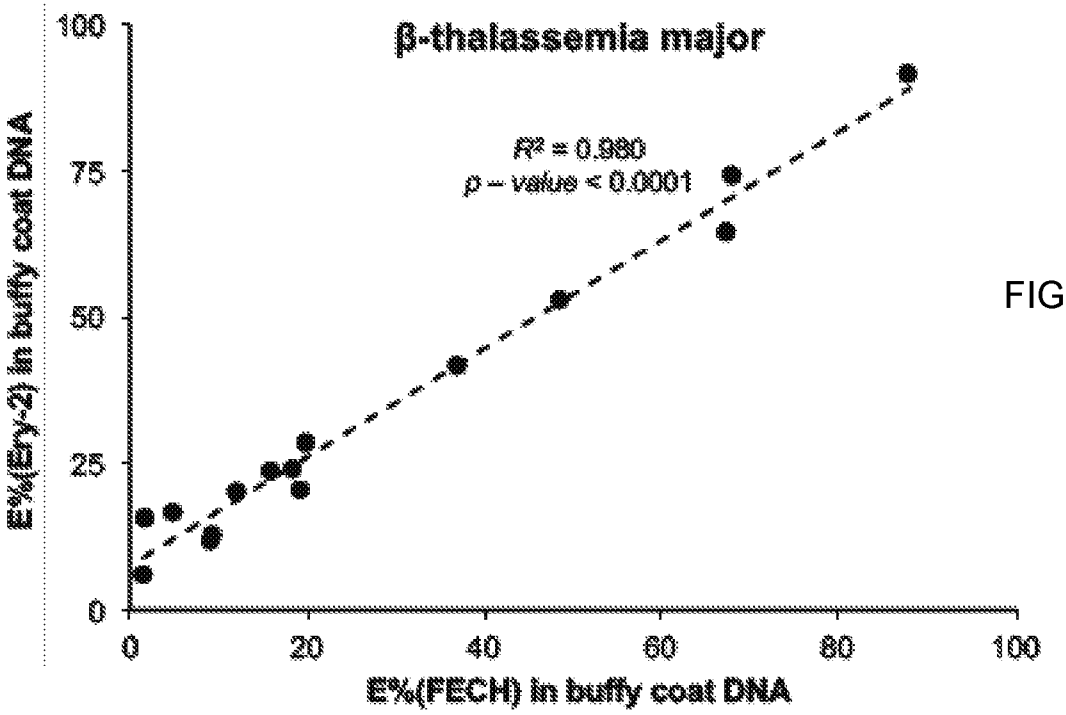

FIG. 18B

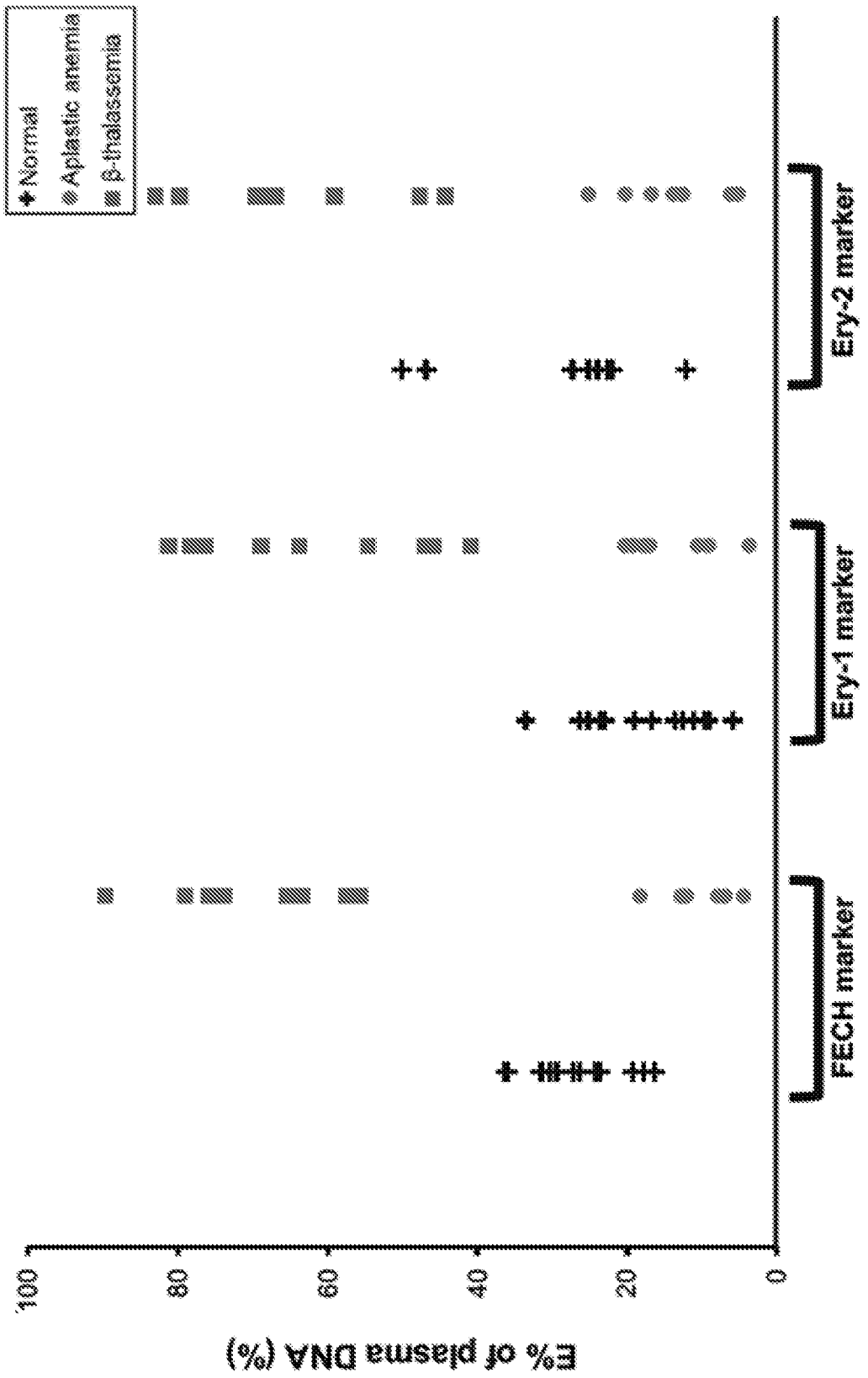
Normal
Aplastic anemia
β-thalassemia
FECH marker
Ery-1 marker
Ery-2 marker
0
20
40
60
80
100
E% of plasma DNA (%)

FIG. 19

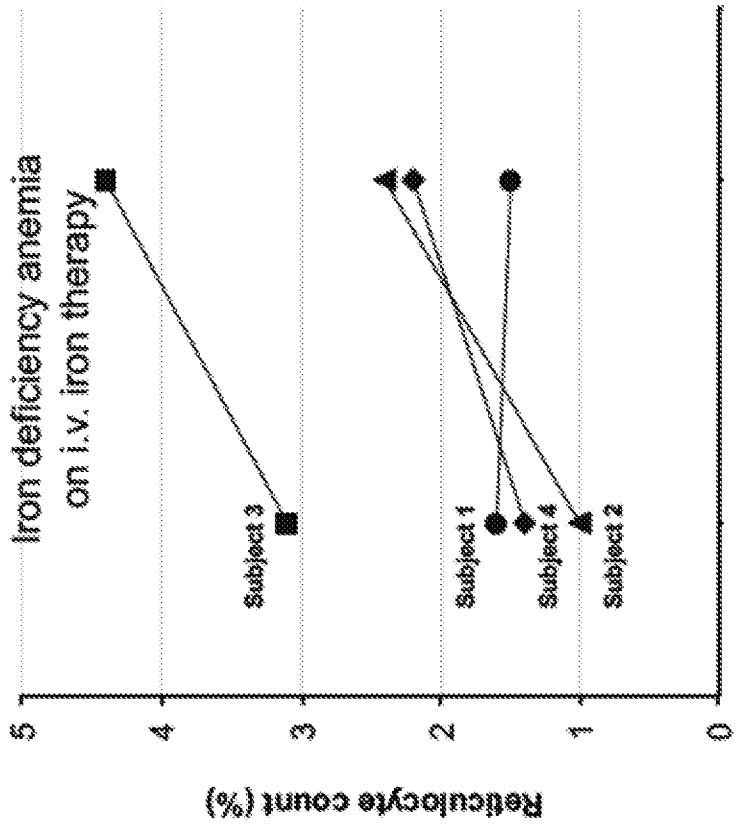

FIG. 20B

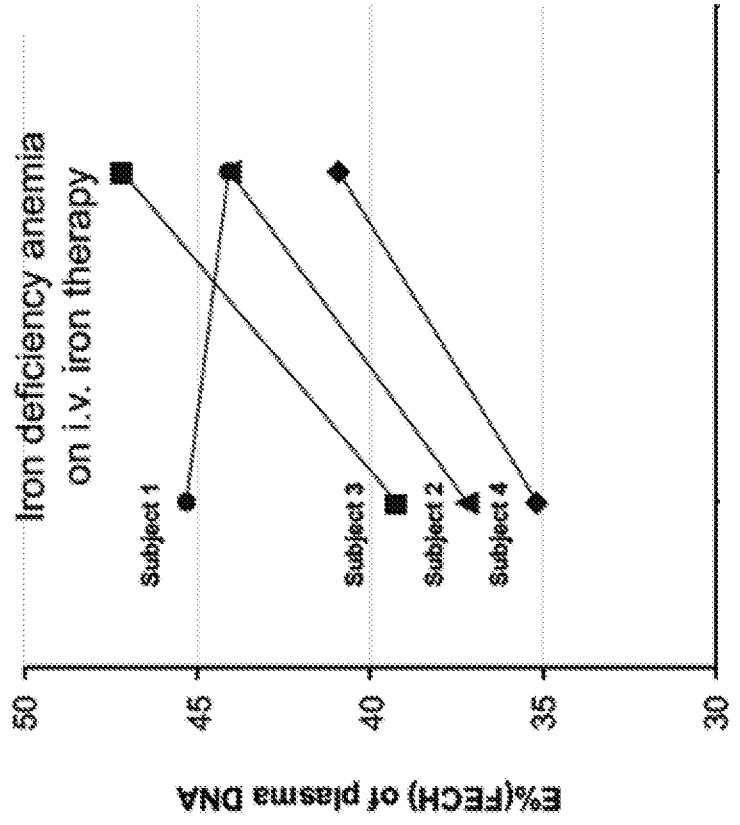

FIG. 20A

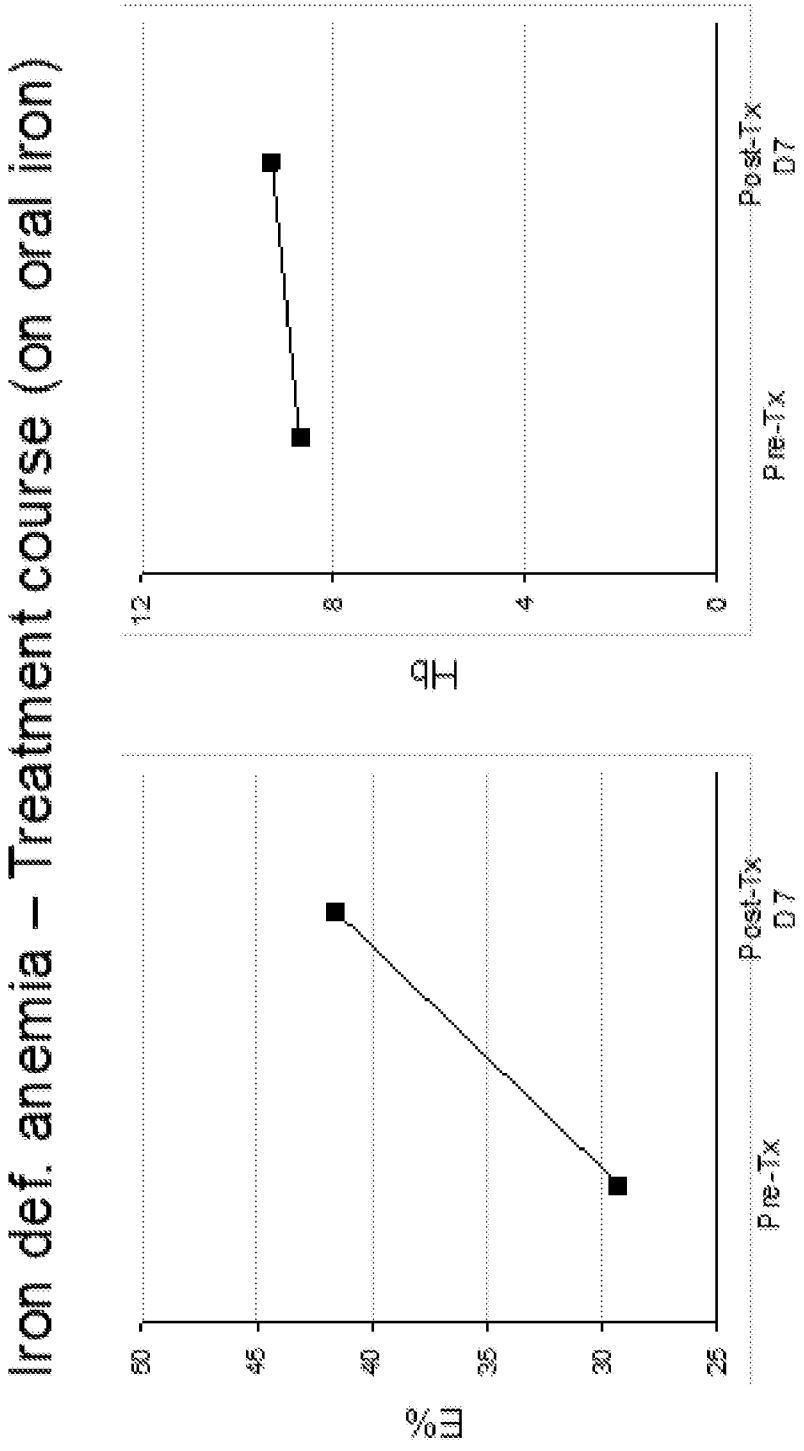

FIG. 21A

FIG. 21B

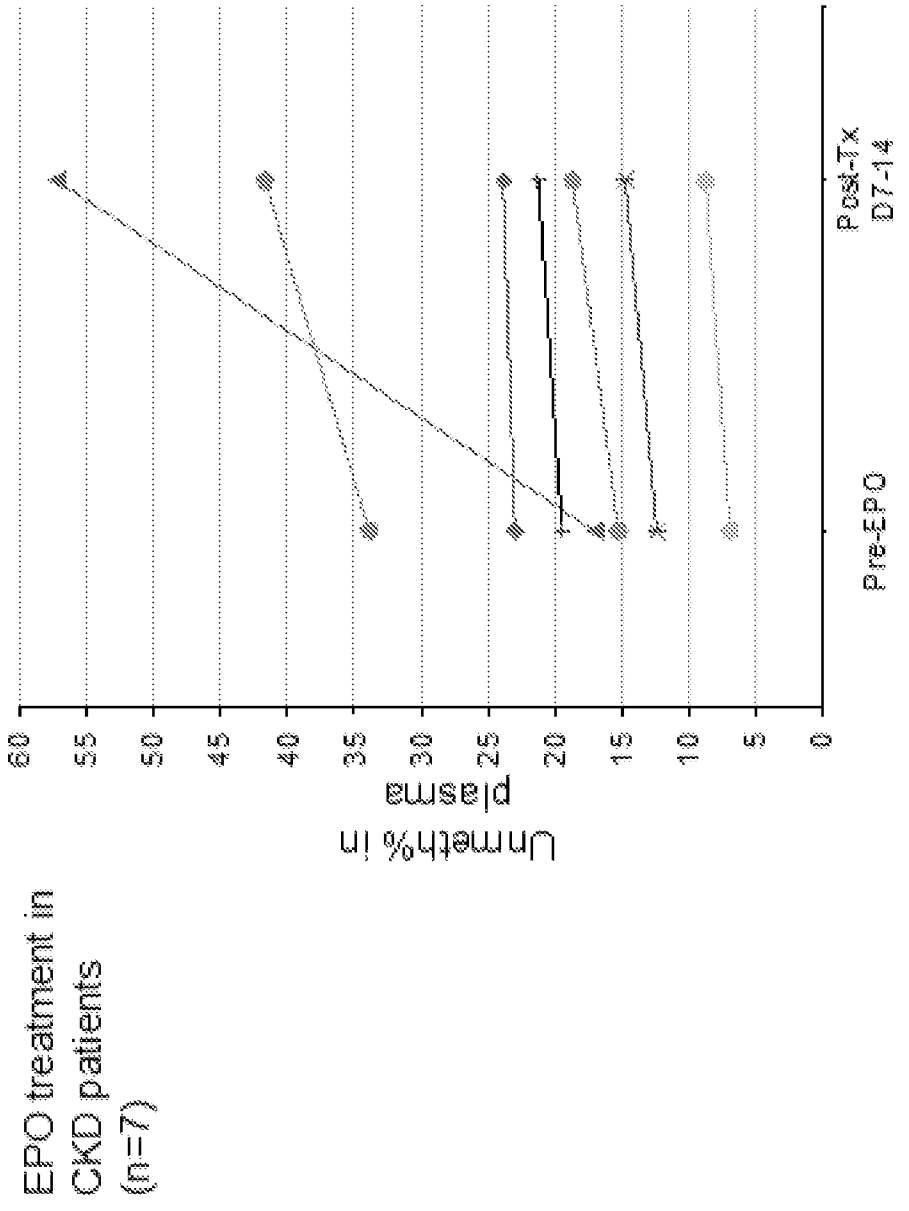
EPO treatment in
CKD patients
(n=7)
Unmeth% in
plasma
60
55
50
45
40
35
30
25
20
15
10
5
0
Pre-EPO
Post-Tx
D7-14

FIG. 22

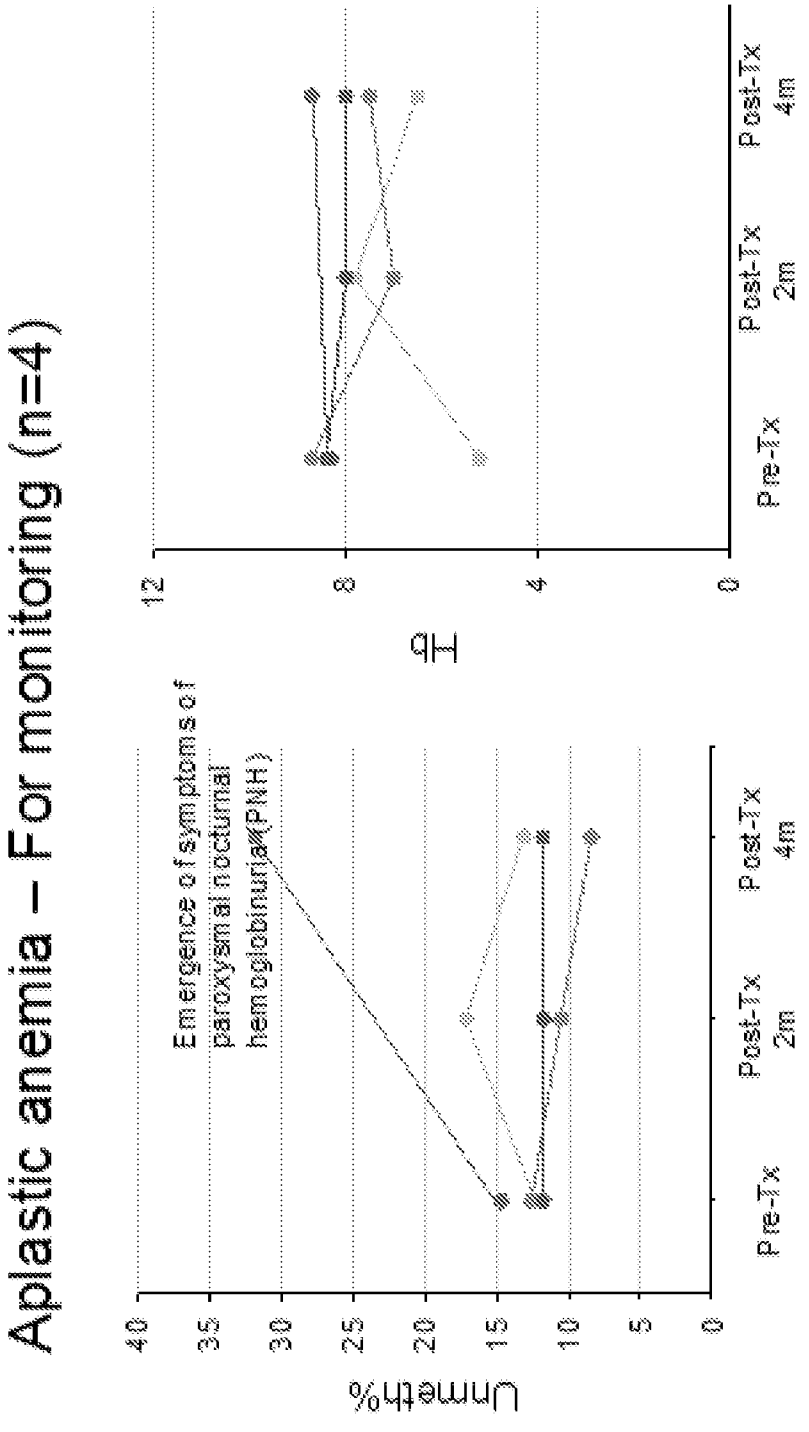

FIG. 23A

FIG. 23B

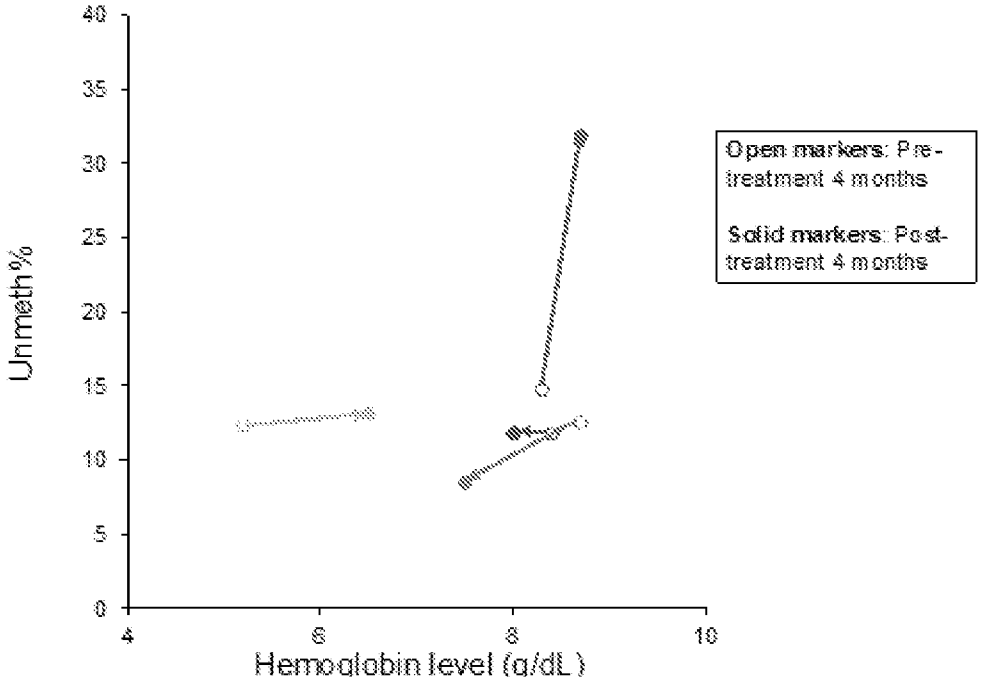

FIG. 24A

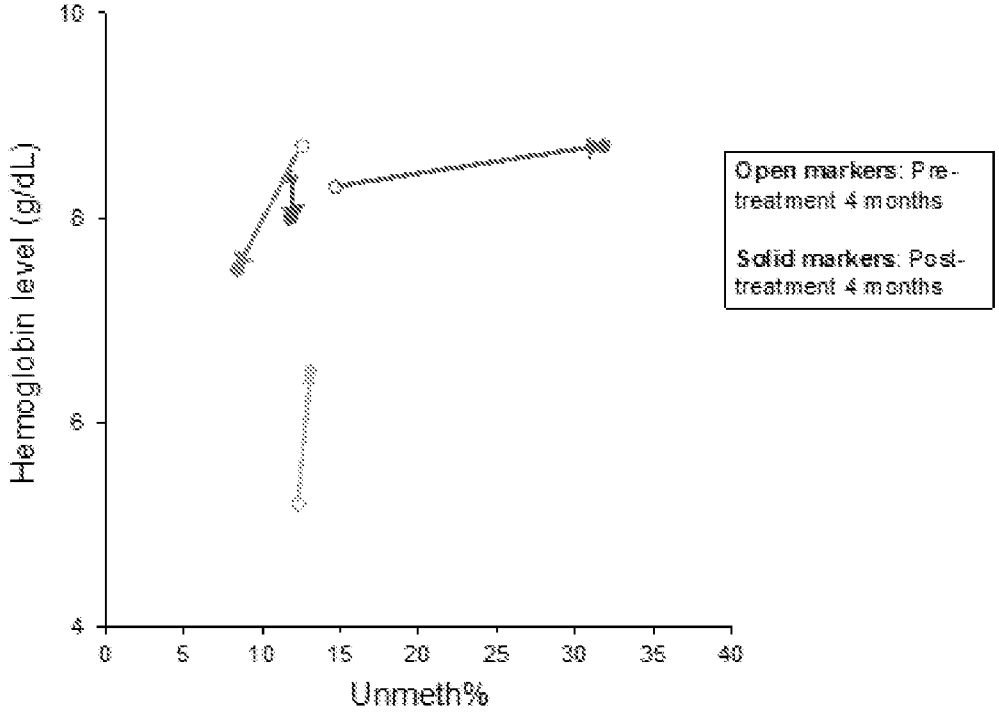

FIG. 24B

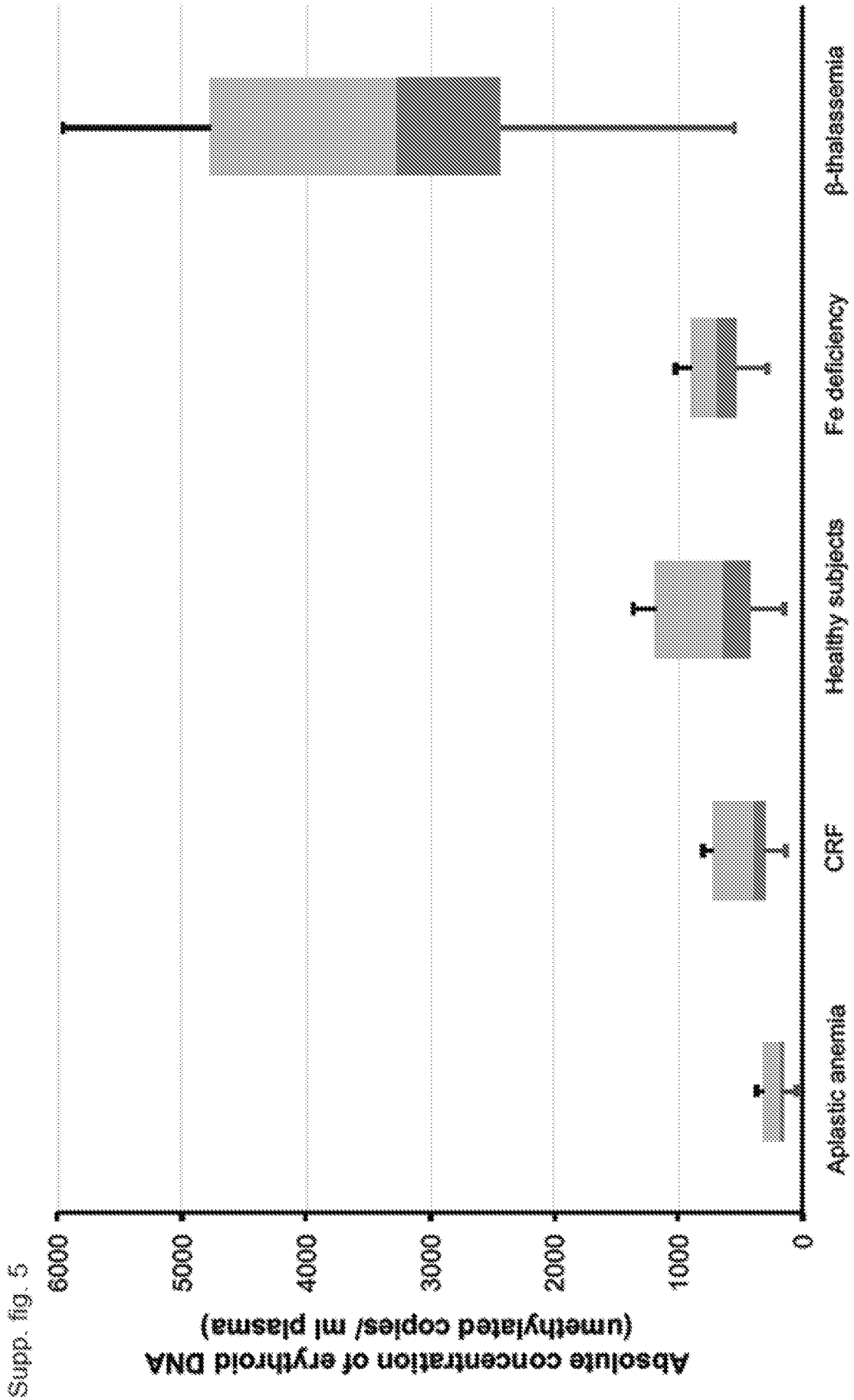
Supp. fig. 5
Absolute concentration of erythroid DNA
(unmethylated copies/ ml plasma)
6000
5000
4000
3000
2000
1000
0
Aplastic anemia
CRF
Healthy subjects
Fe deficiency
β-thalassemia

FIG. 25

2600

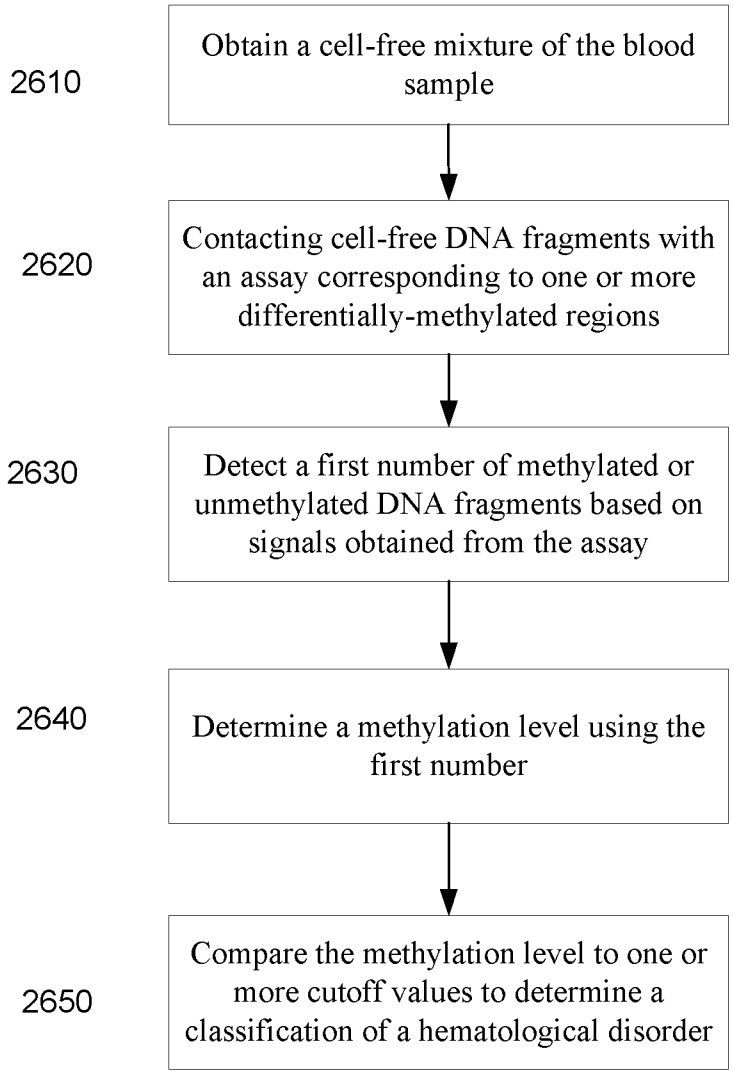
2610
Obtain a cell-free mixture of the blood sample
2620
Contacting cell-free DNA fragments with an assay corresponding to one or more differentially-methylated regions
2630
Detect a first number of methylated or unmethylated DNA fragments based on signals obtained from the assay
2640
Determine a methylation level using the first number
2650
Compare the methylation level to one or more cutoff values to determine a classification of a hematological disorder

FIG. 26

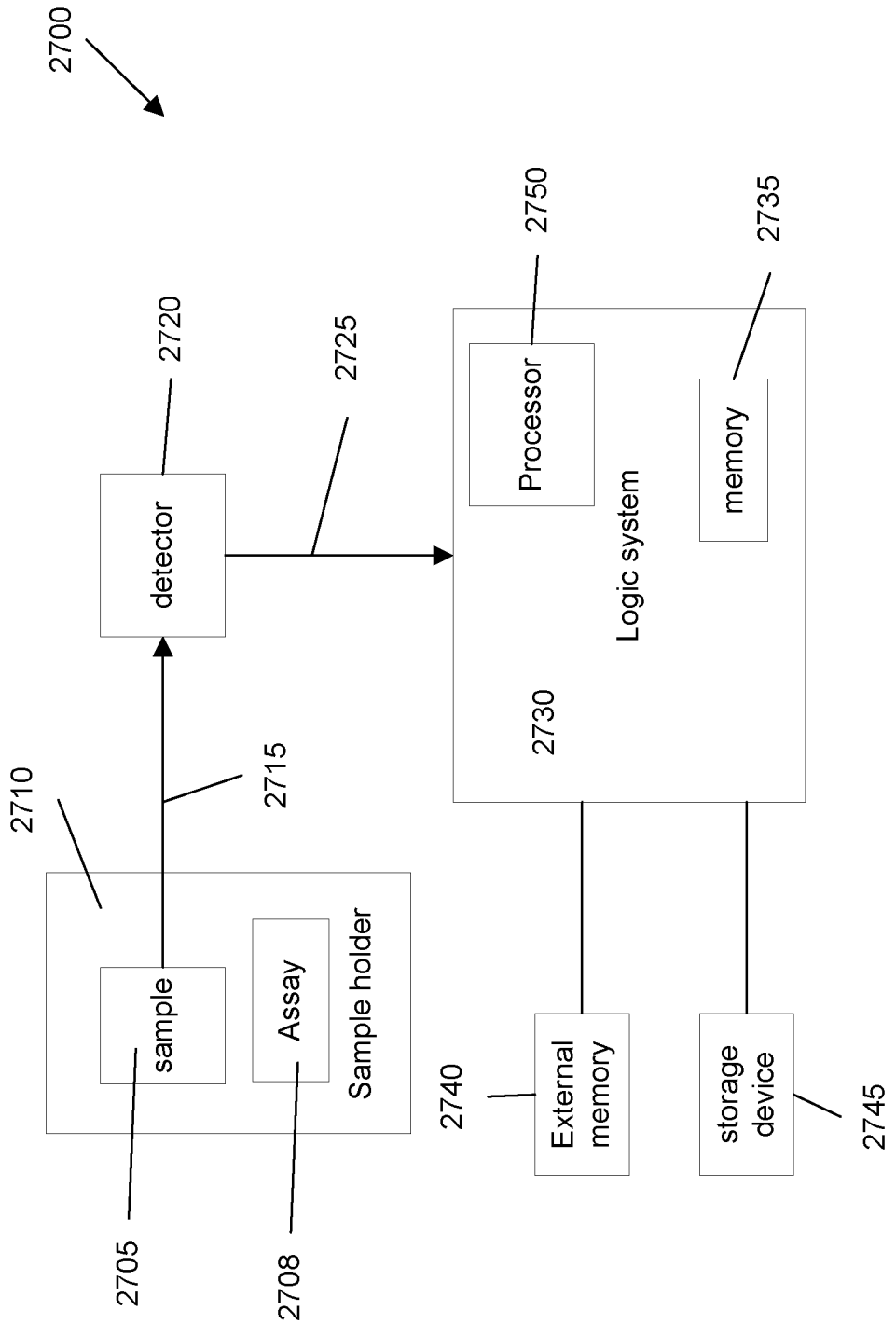
2700
2720
2725
2750
2735
detector
Processor
memory
Logic system
2730
2710
2715
sample
Assay
Sample holder
2740
External memory
storage device
2745
2705
2708

FIG. 27

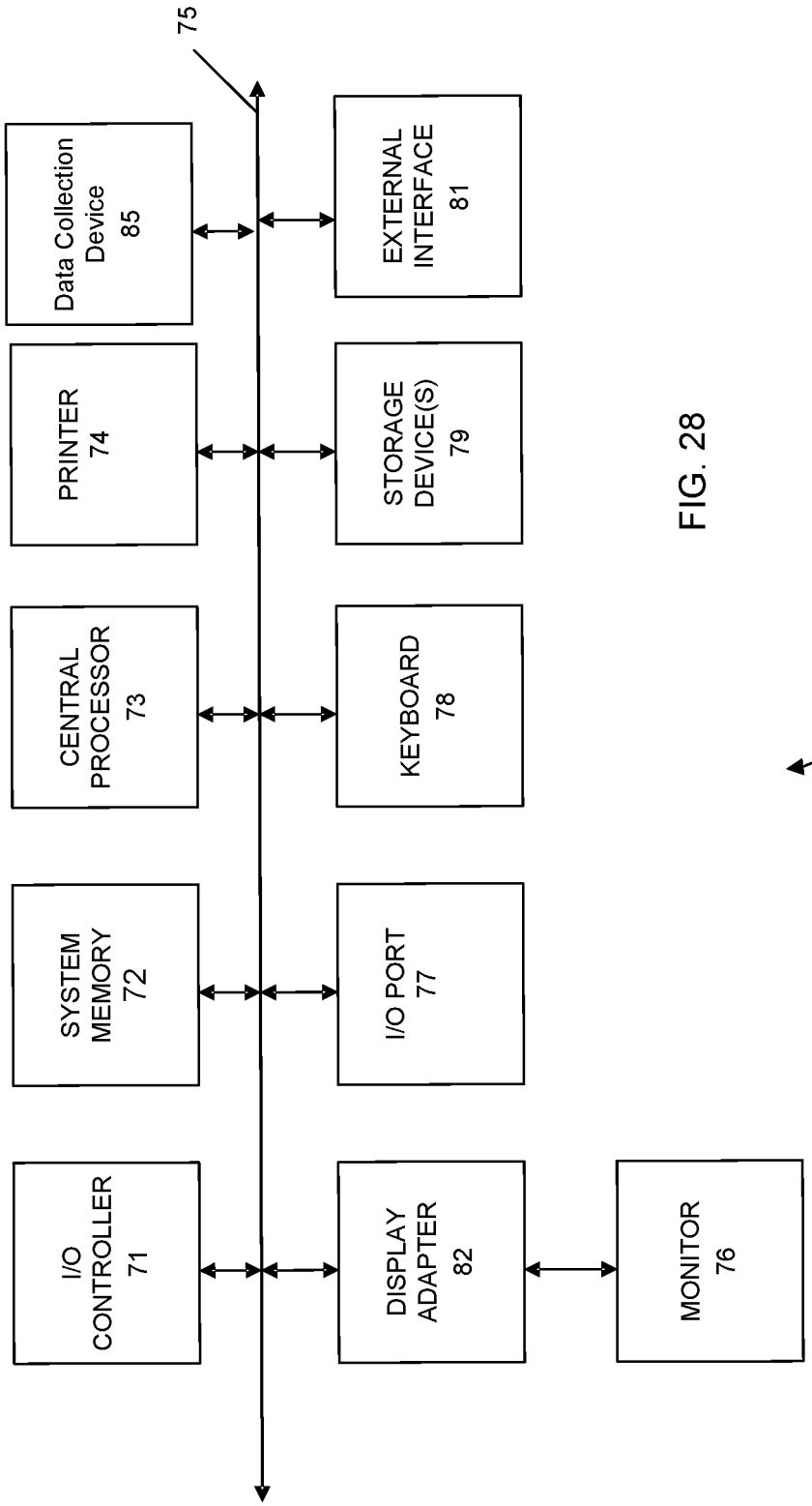
75
Data Collection Device 85
EXTERNAL INTERFACE 81
PRINTER 74
STORAGE DEVICE(S) 79
CENTRAL PROCESSOR 73
KEYBOARD 78
SYSTEM MEMORY 72
I/O PORT 77
I/O CONTROLLER 71
DISPLAY ADAPTER 82
MONITOR 76
10

FIG. 28