FIGURE 1A

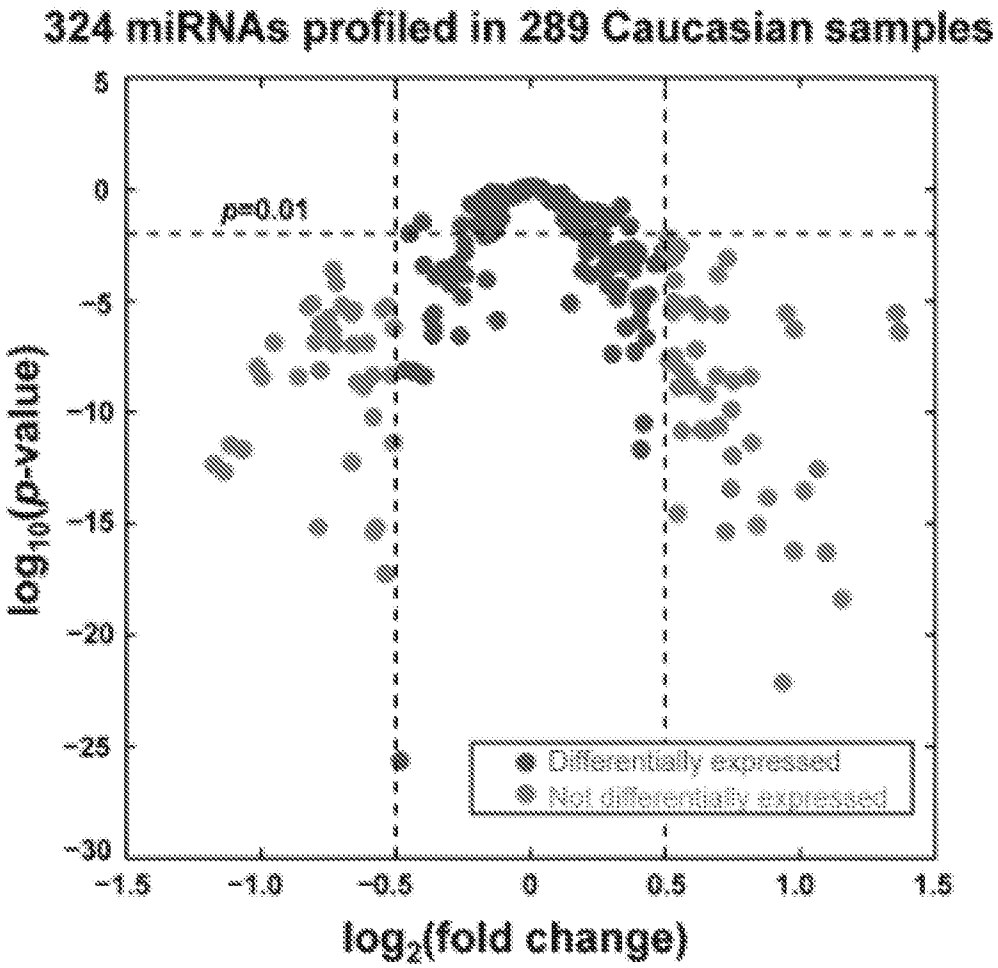

324 miRNAs profiled in 289 Caucasian samples
p=0.01
log10(p-value)
log2(fold change)
Differentially expressed
Not differentially expressed
5
0
−5
−10
−15
−20
−25
−30
−1.5
−1.0
−0.5
0
0.5
1.0
1.5

FIGURE 1B

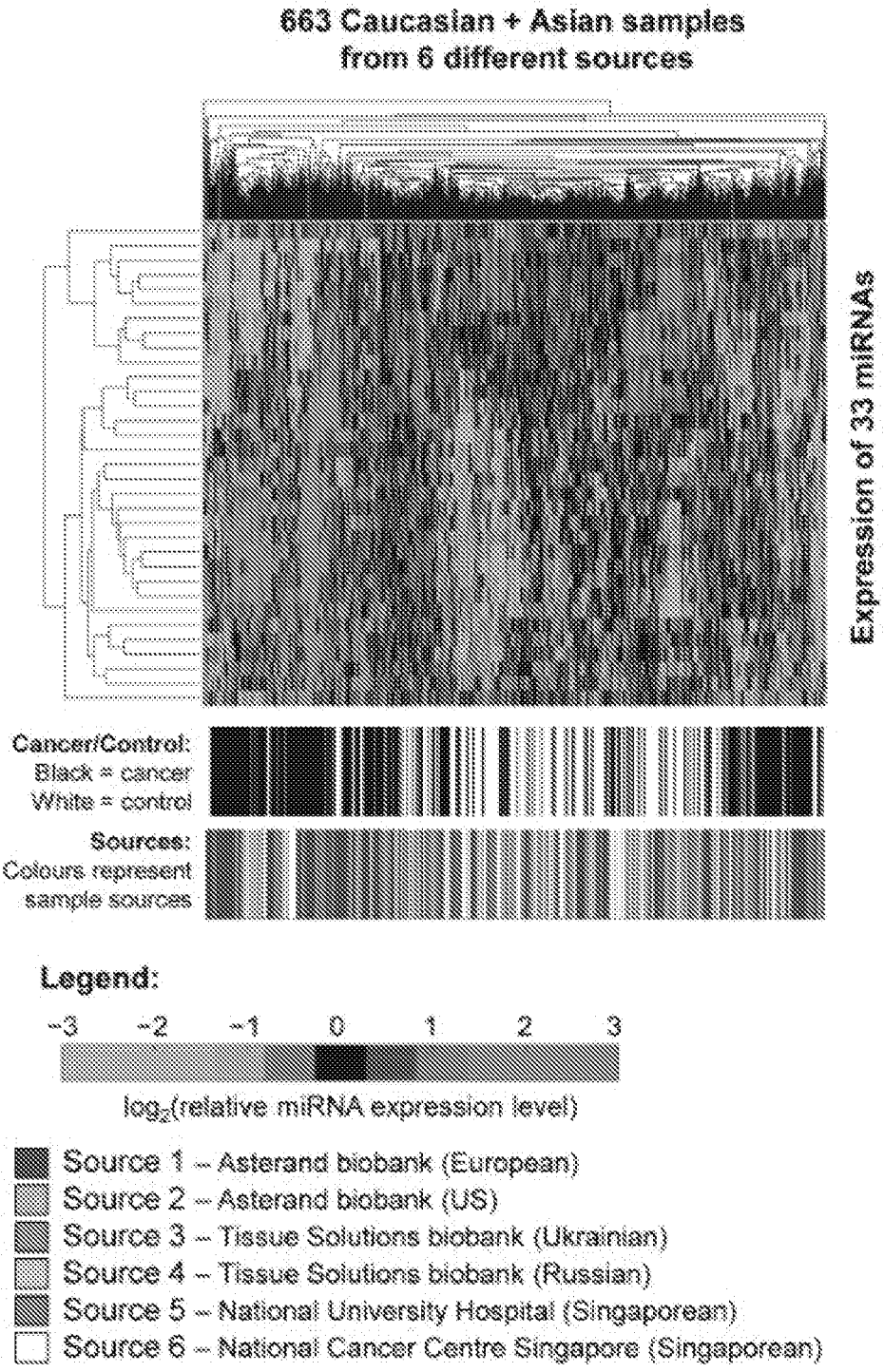

663 Caucasian + Asian samples
from 6 different sources
Expression of 33 miRNAs
Cancer/Control:
Black = cancer
White = control
Sources:
Colours represent
sample sources
Legend:
−3 −2 −1 0 1 2 3
log2(relative miRNA expression level)
Source 1 – Asterand biobank (European)
Source 2 – Asterand biobank (US)
Source 3 – Tissue Solutions biobank (Ukrainian)
Source 4 – Tissue Solutions biobank (Russian)
Source 5 – National University Hospital (Singaporean)
Source 6 – National Cancer Centre Singapore (Singaporean)

FIGURE 1C

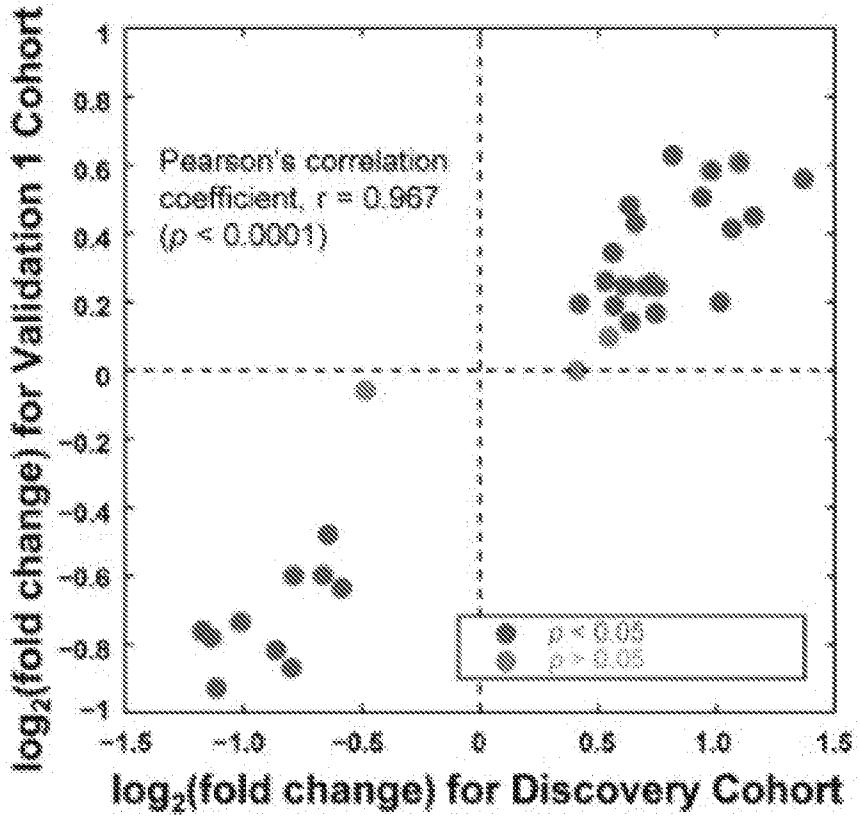

Pearson's correlation coefficient, r = 0.967
(p < 0.0001)
p < 0.05
p > 0.05
log2(fold change) for Validation 1 Cohort
log2(fold change) for Discovery Cohort
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1
−1.5
−1.0
−0.5
0
0.5
1.0
1.5

FIGURE 2A

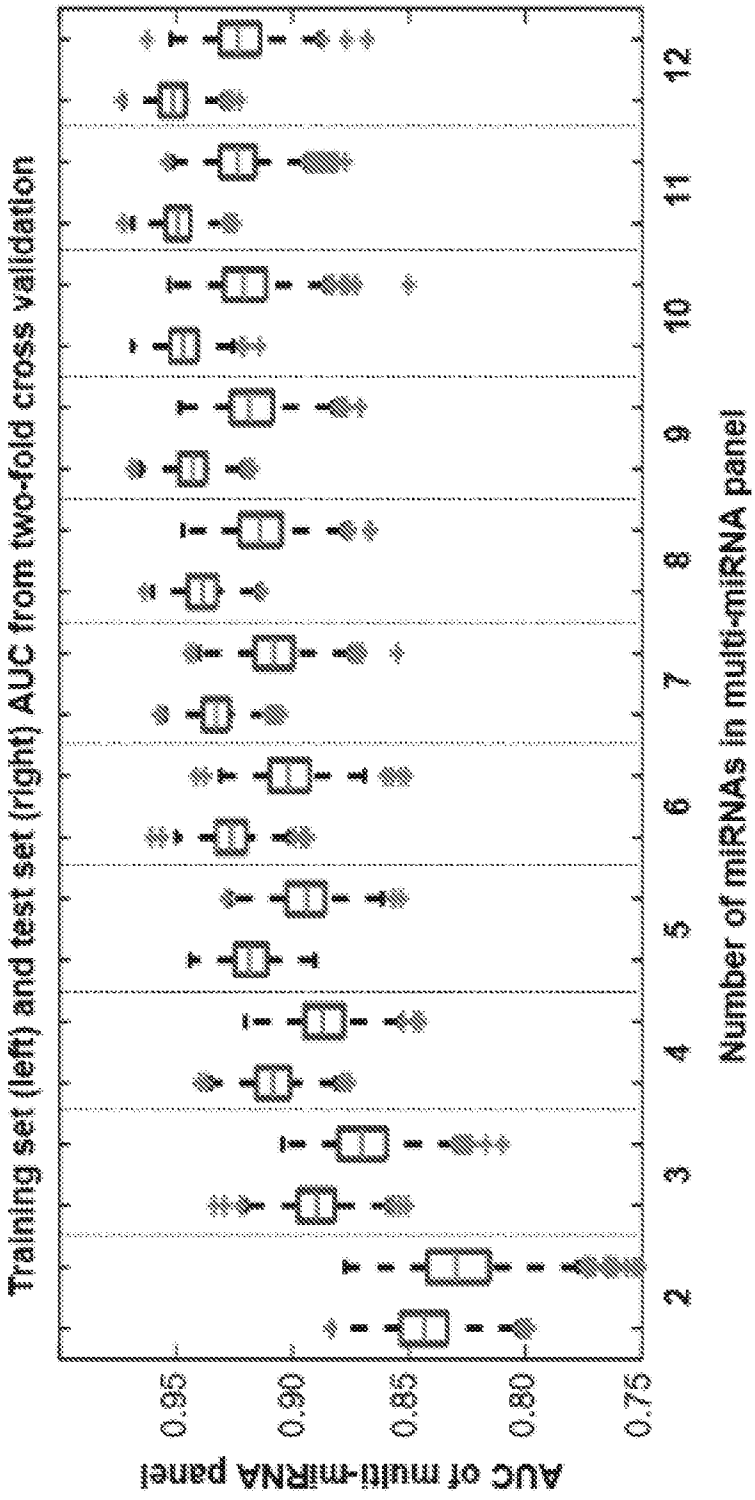

Training set (left) and test set (right) AUC from two-fold cross validation
AUC of multi-miRNA panel
0.95
0.90
0.85
0.80
0.75
2
3
4
5
6
7
8
9
10
11
12
Number of miRNAs in multi-miRNA panel

FIGURE 2B

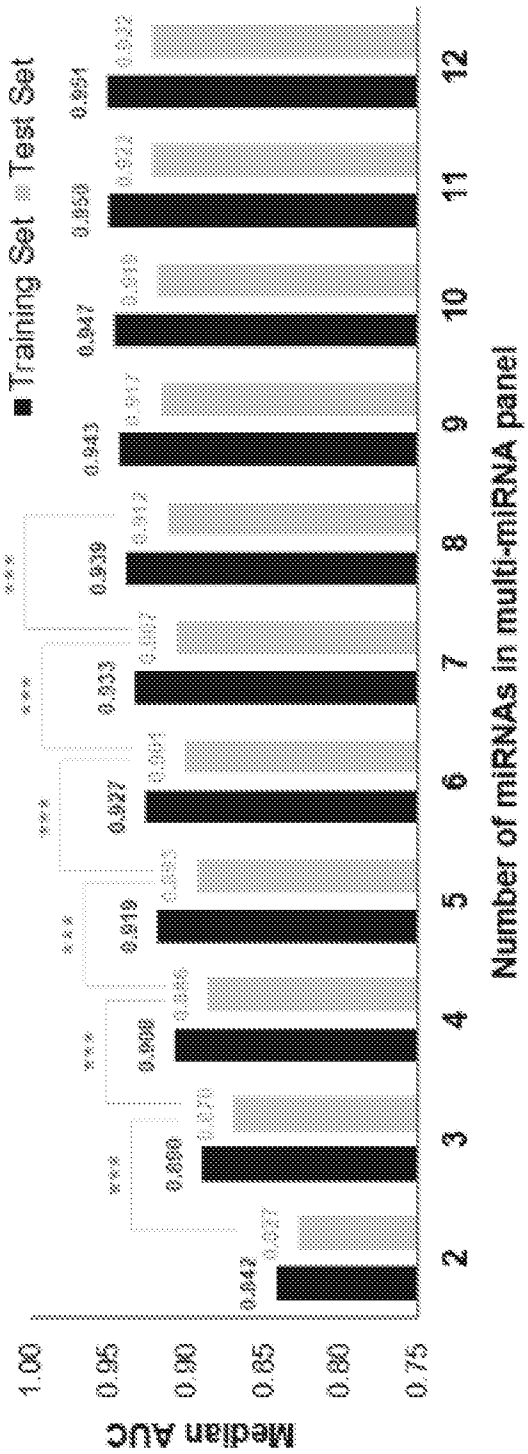

Training Set
Test Set
Median AUC
1.00
0.95
0.90
0.85
0.80
0.75
2
3
4
5
6
7
8
9
10
11
12
Number of miRNAs in multi-miRNA panel
***
0.843
0.877
0.880
0.870
0.898
0.886
0.916
0.893
0.927
0.901
0.933
0.907
0.939
0.913
0.943
0.917
0.947
0.919
0.950
0.921
0.951
0.922

FIGURE 2C

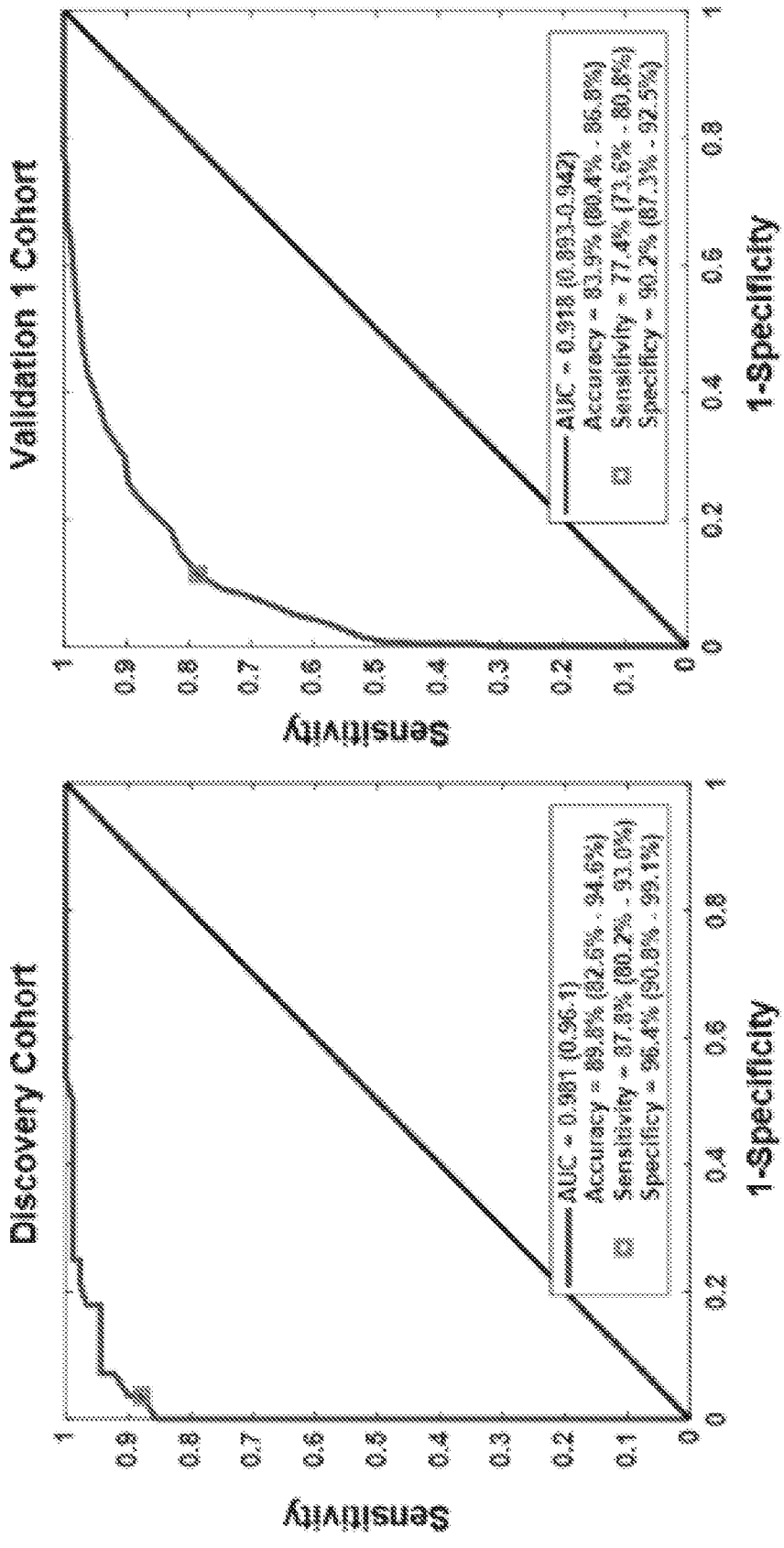

Validation 1 Cohort
Sensitivity
1-Specificity
AUC = 0.918 (0.883-0.942)
Accuracy = 83.9% (80.4% - 86.8%)
Sensitivity = 77.4% (73.6% - 80.8%)
Specificity = 90.2% (87.3% - 92.5%)
Discovery Cohort
Sensitivity
1-Specificity
AUC = 0.981 (0.96-1)
Accuracy = 89.8% (82.6% - 94.6%)
Sensitivity = 87.8% (80.2% - 93.0%)
Specificity = 96.4% (90.8% - 99.1%)

FIGURE 3A

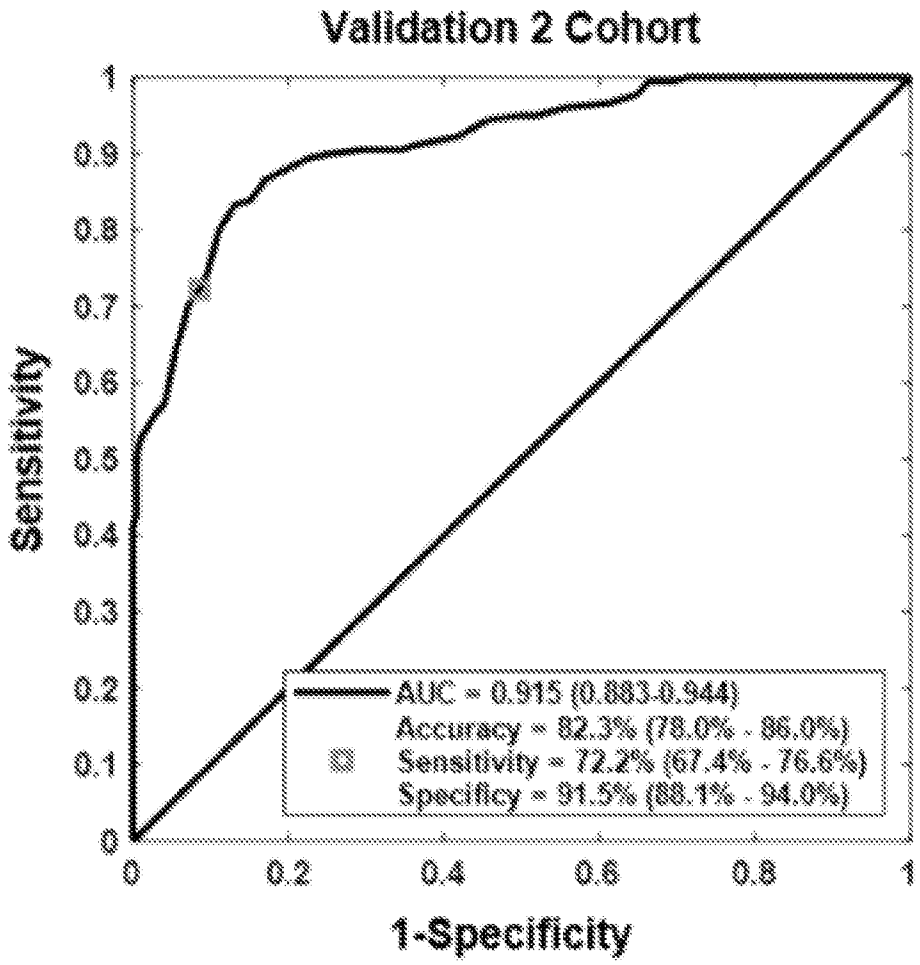

Validation 2 Cohort
Sensitivity
1-Specificity
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1
AUC = 0.915 (0.883-0.944)
Accuracy = 82.3% (78.0% - 86.0%)
Sensitivity = 72.2% (67.4% - 76.6%)
Specificy = 91.5% (88.1% - 94.0%)

FIGURE 3B

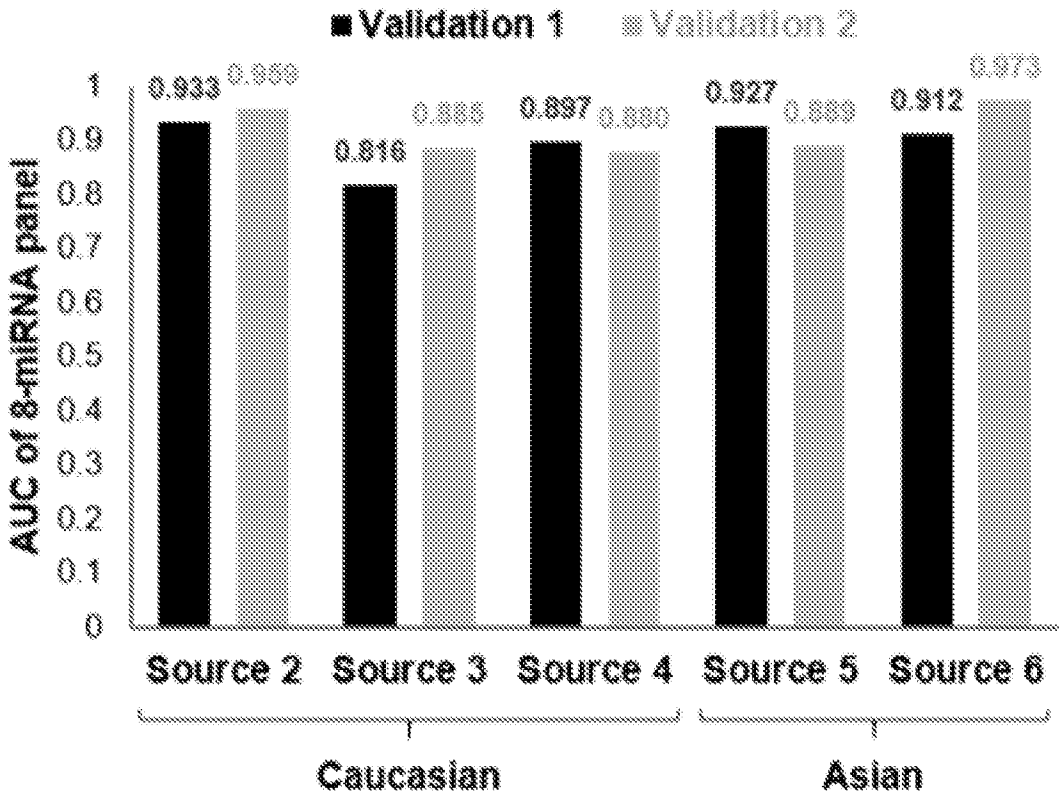

Validation 1
Validation 2
AUC of 8-miRNA panel
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.933
0.959
0.816
0.885
0.897
0.880
0.927
0.889
0.912
0.973
Source 2
Source 3
Source 4
Source 5
Source 6
Caucasian
Asian

FIGURE 3C

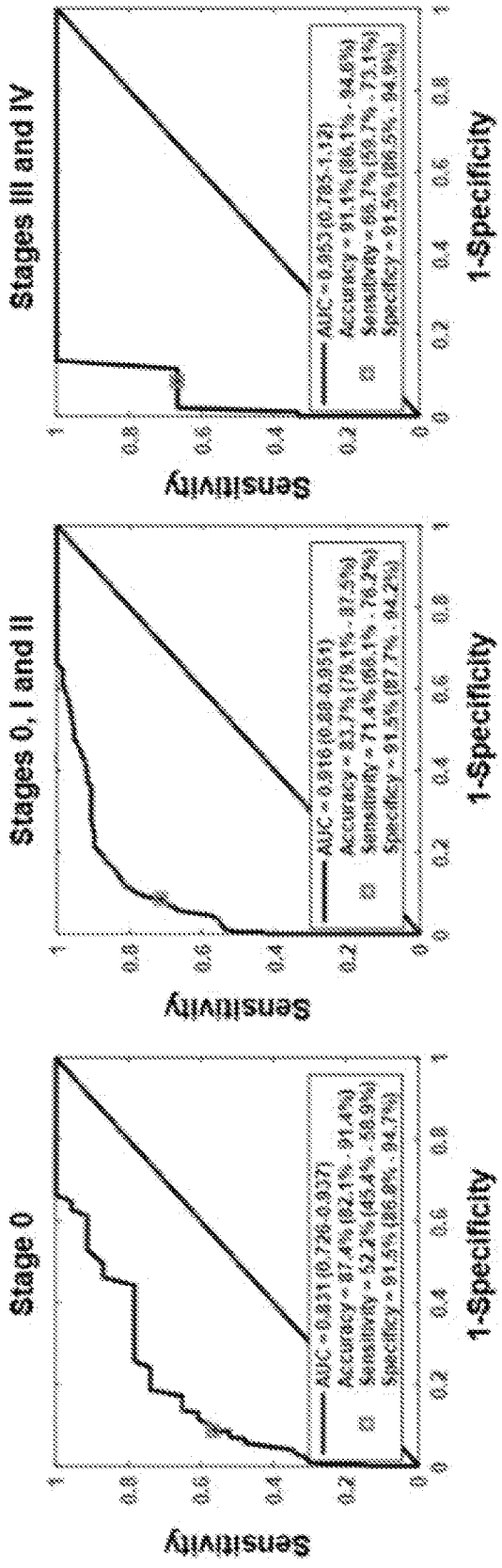

Stages III and IV
Sensitivity
1-Specificity
AUC = 0.853 (0.785-1.12)
Accuracy = 91.1% (86.1% - 94.8%)
Sensitivity = 66.7% (50.7% - 73.1%)
Specificity = 91.5% (86.5% - 94.9%)
Stages 0, I and II
Sensitivity
1-Specificity
AUC = 0.816 (0.68-0.951)
Accuracy = 83.7% (78.1% - 87.9%)
Sensitivity = 71.4% (66.1% - 76.3%)
Specificity = 91.5% (87.7% - 94.2%)
Stage 0
Sensitivity
1-Specificity
AUC = 0.831 (0.726-0.937)
Accuracy = 87.4% (82.1% - 91.4%)
Sensitivity = 52.2% (43.4% - 58.9%)
Specificity = 91.5% (86.8% - 94.7%)

FIGURE 4A

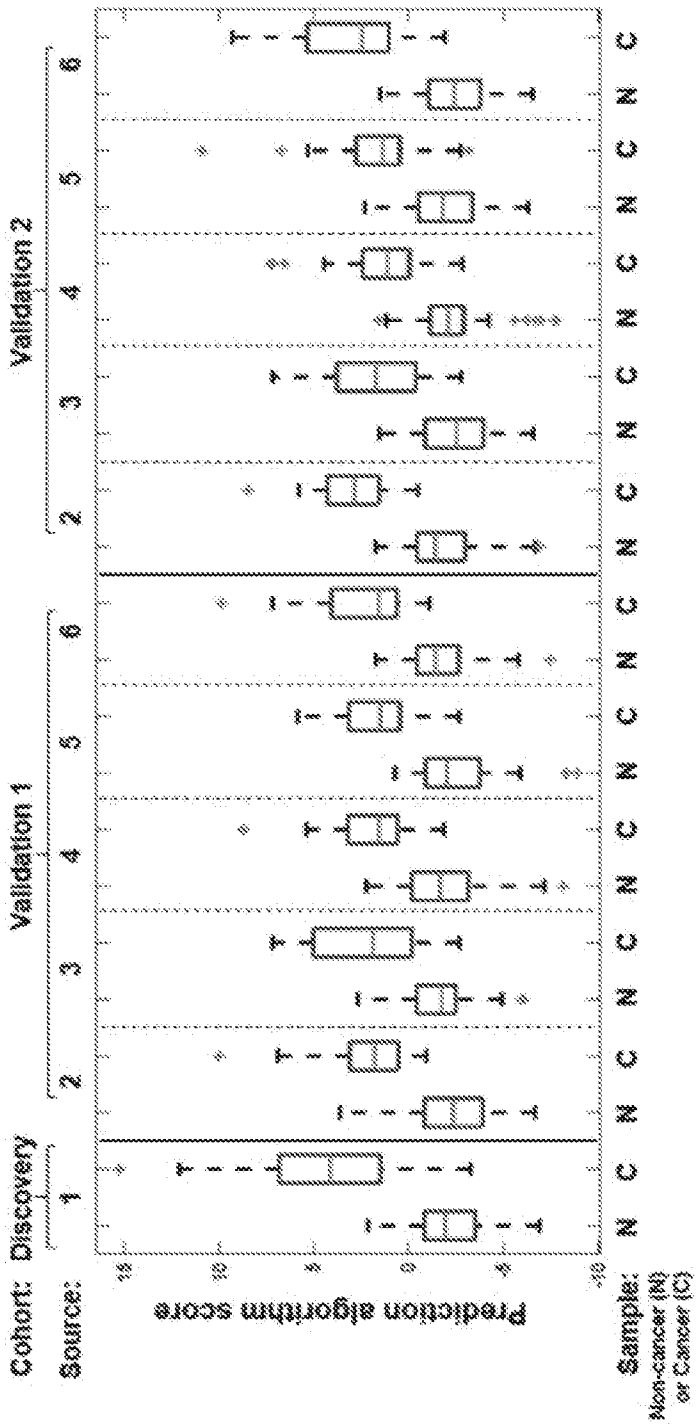

Cohort:
Discovery
Validation 1
Validation 2
Source:
1
2
3
4
5
6
Prediction algorithm score
Sample:
Non-cancer (N)
or Cancer (C)
N
C

FIGURE 4B

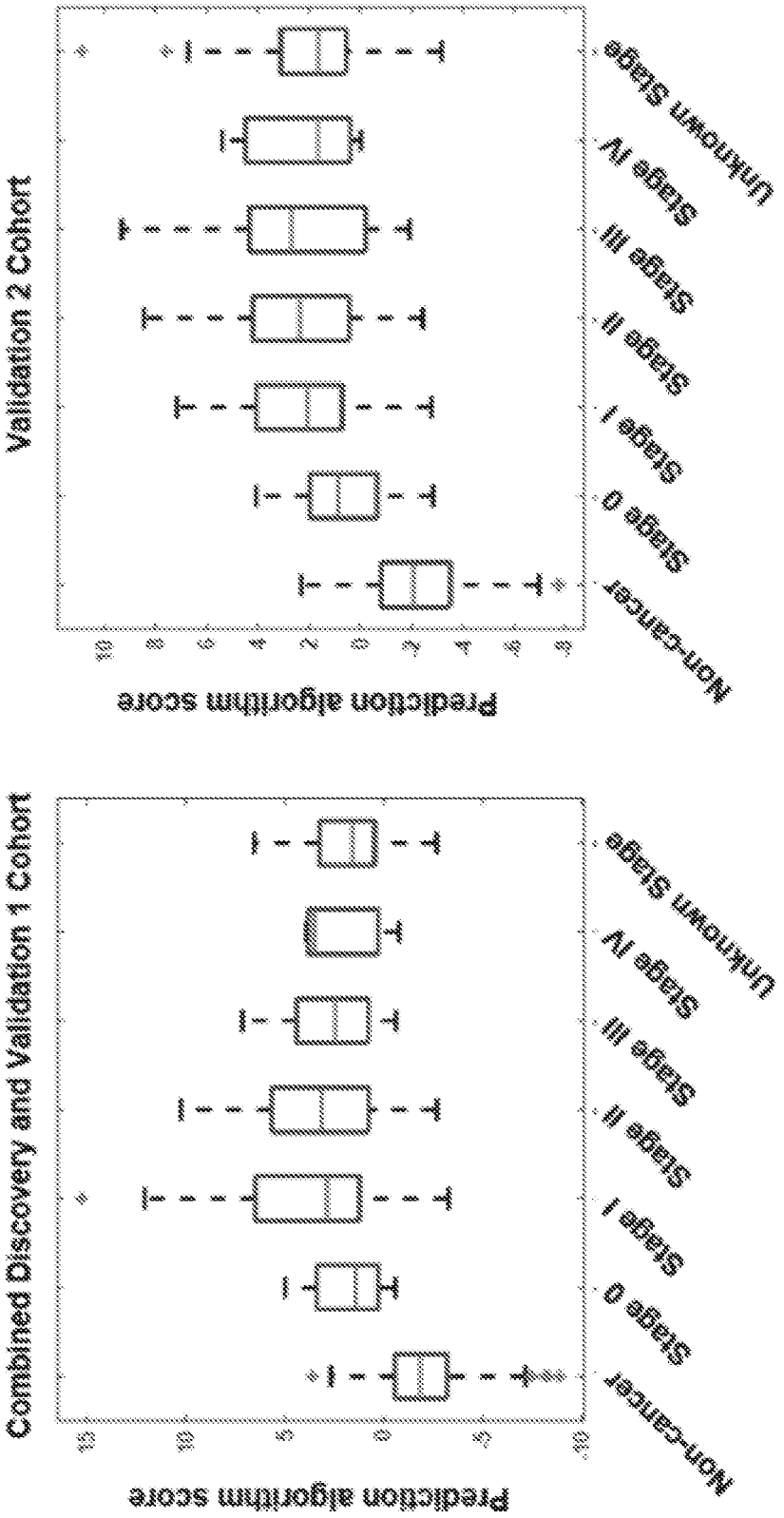

Validation 2 Cohort
Prediction algorithm score
Non-cancer
Stage 0
Stage I
Stage II
Stage III
Stage IV
Unknown Stage
Combined Discovery and Validation 1 Cohort
Prediction algorithm score
Non-cancer
Stage 0
Stage I
Stage II
Stage III
Stage IV
Unknown Stage

FIGURE 5

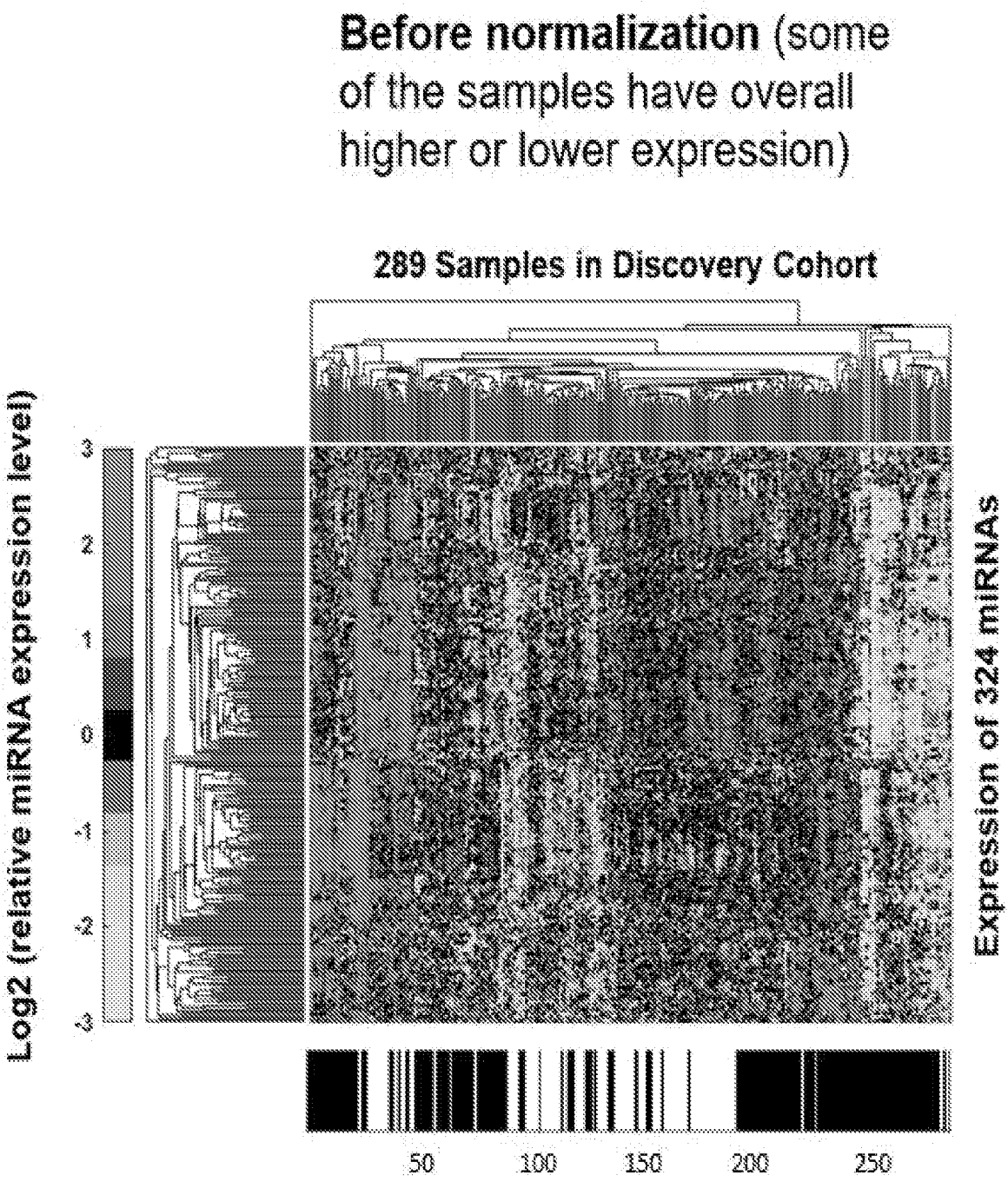

Before normalization (some of the samples have overall higher or lower expression)
289 Samples in Discovery Cohort
Log2 (relative miRNA expression level)
3
2
1
0
-1
-2
-3
Expression of 324 miRNAs
50
100
150
200
250

FIGURE 5 continued

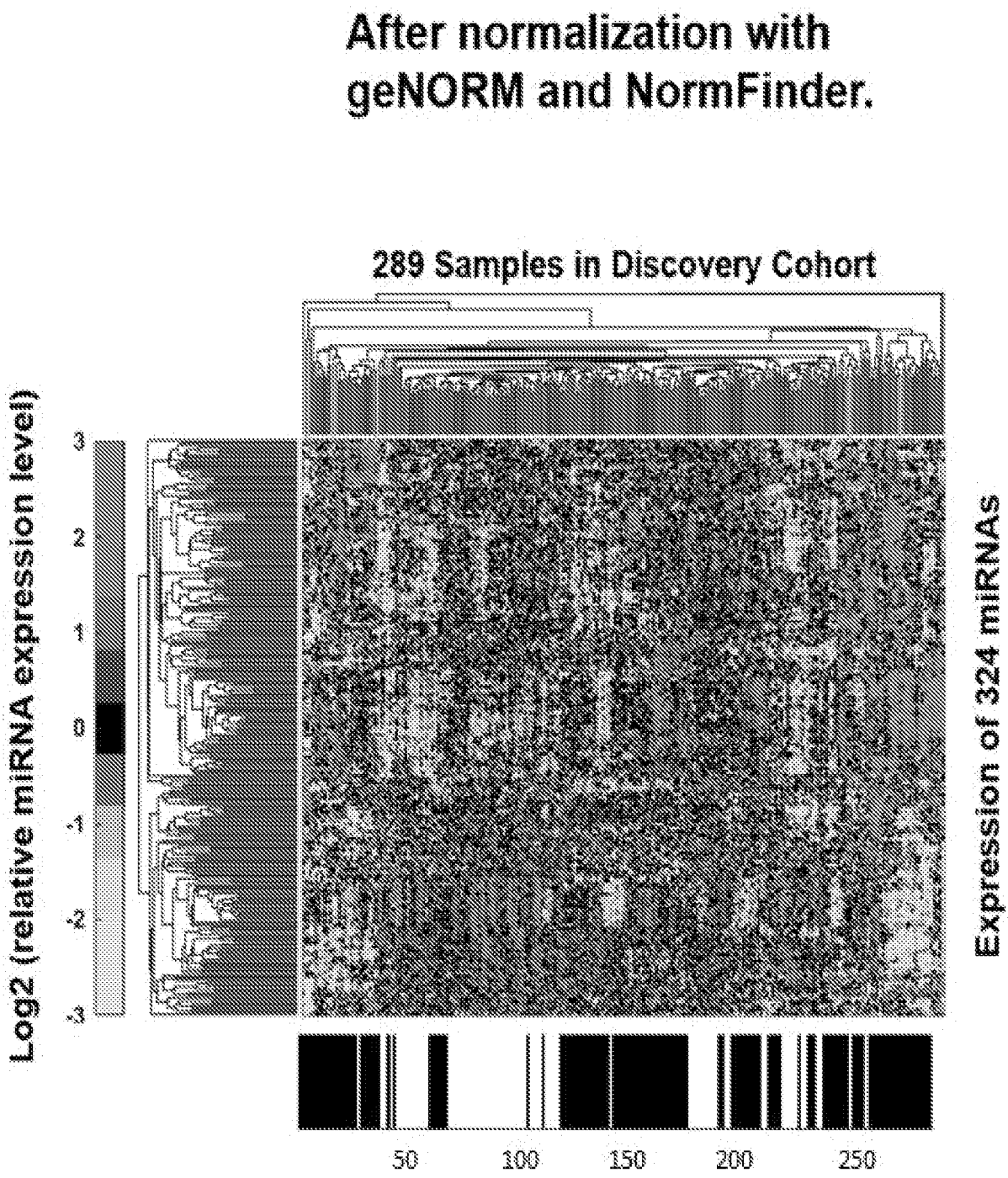
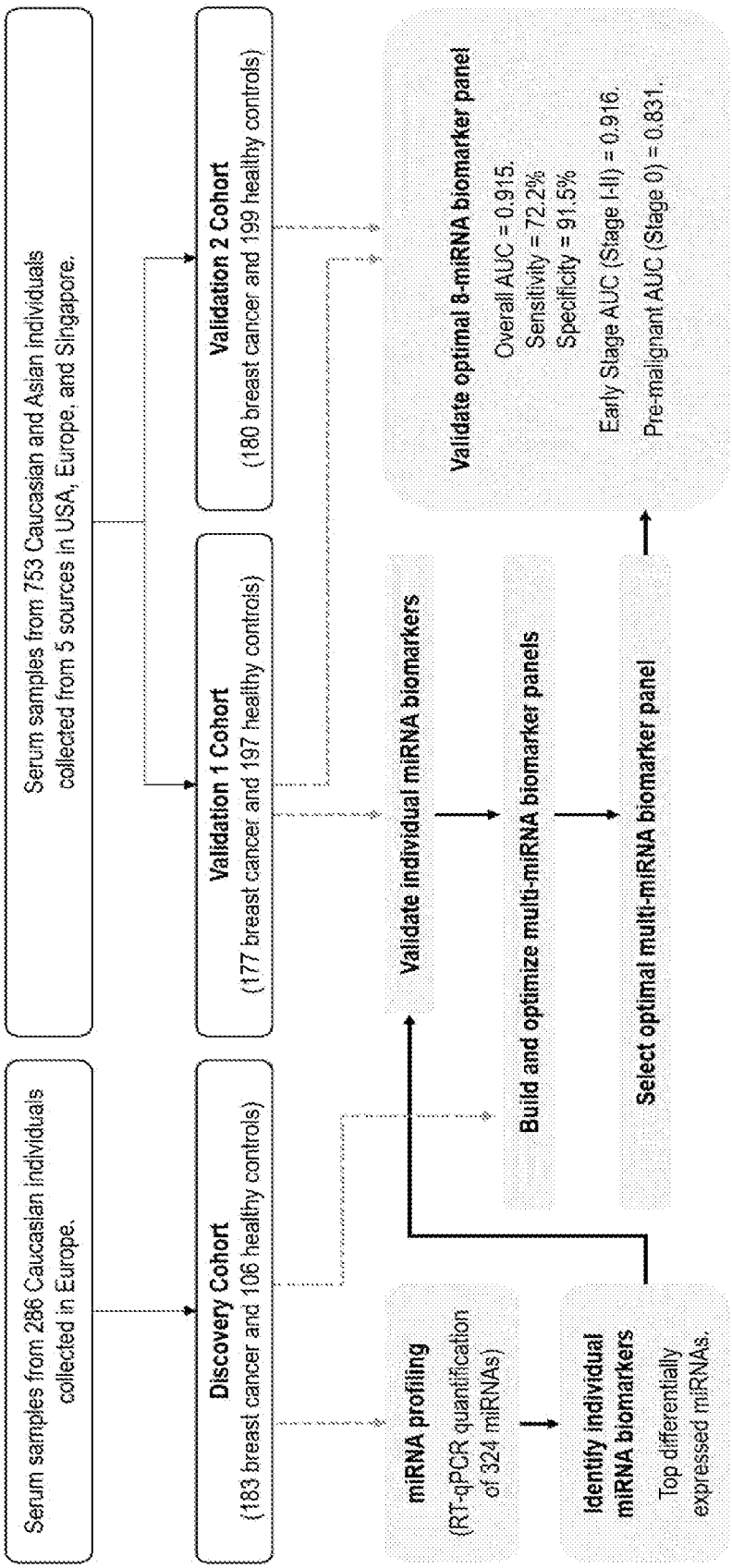


FIGURE 6


Serum samples from 753 Caucasian and Asian individuals collected from 5 sources in USA, Europe, and Singapore.
Serum samples from 286 Caucasian individuals collected in Europe.
Discovery Cohort
(183 breast cancer and 106 healthy controls)
Validation 1 Cohort
(177 breast cancer and 197 healthy controls)
Validation 2 Cohort
(180 breast cancer and 199 healthy controls)
miRNA profiling
(RT-qPCR quantification of 324 miRNAs)
Identify individual miRNA biomarkers
Top differentially expressed miRNAs.
Validate individual miRNA biomarkers
Build and optimize multi-miRNA biomarker panels
Select optimal multi-miRNA biomarker panel
Validate optimal 8-miRNA biomarker panel
Overall AUC = 0.915.
Sensitivity = 72.2%
Specificity = 91.5%
Early Stage AUC (Stage I-II) = 0.916.
Pre-malignant AUC (Stage 0) = 0.831.