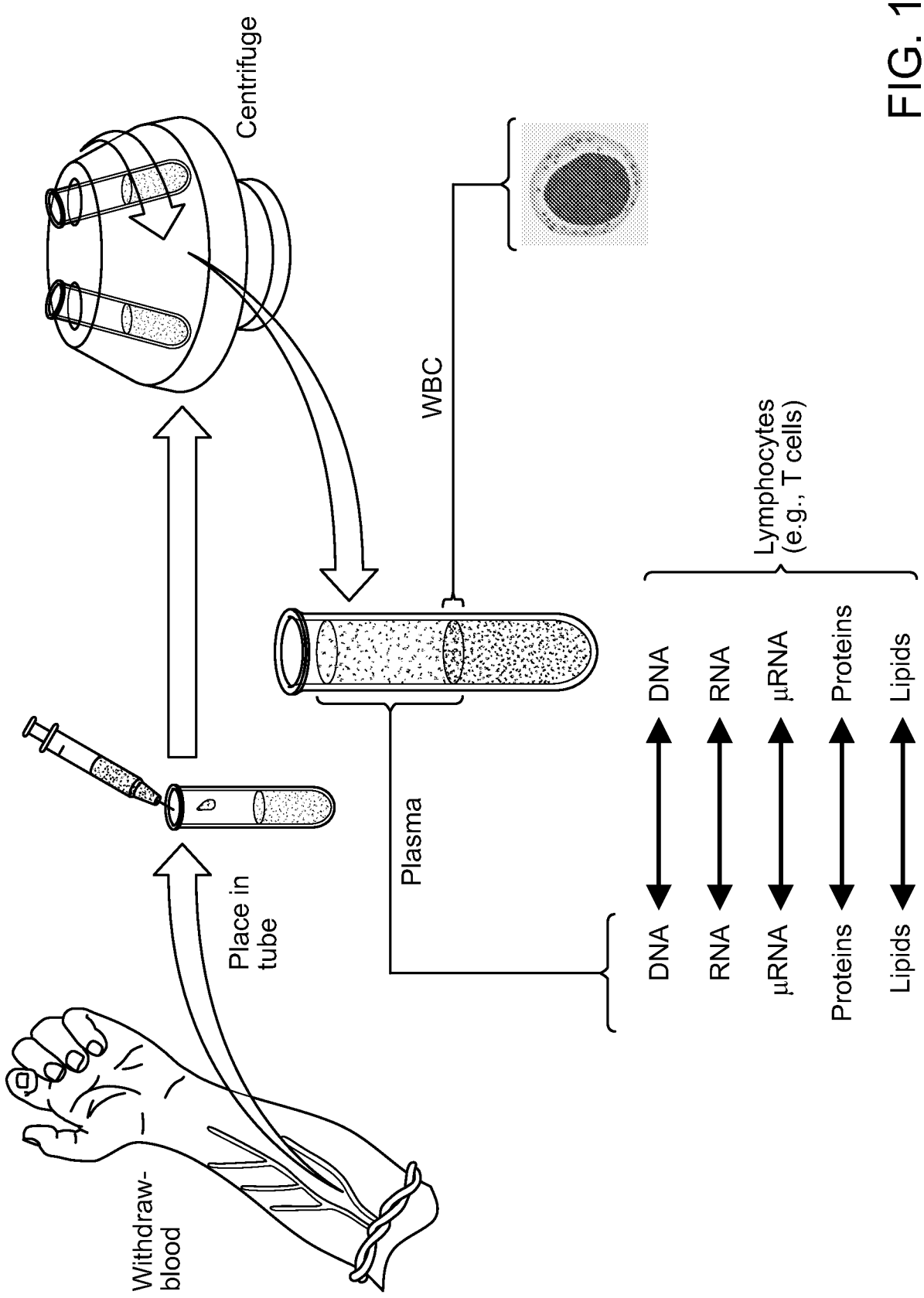
Withdraw-blood
Place in tube
Centrifuge
Plasma
WBC
Lymphocytes (e.g., T cells)
DNA
RNA
µRNA
Proteins
Lipids
DNA
RNA
µRNA
Proteins
Lipids

FIG. 1

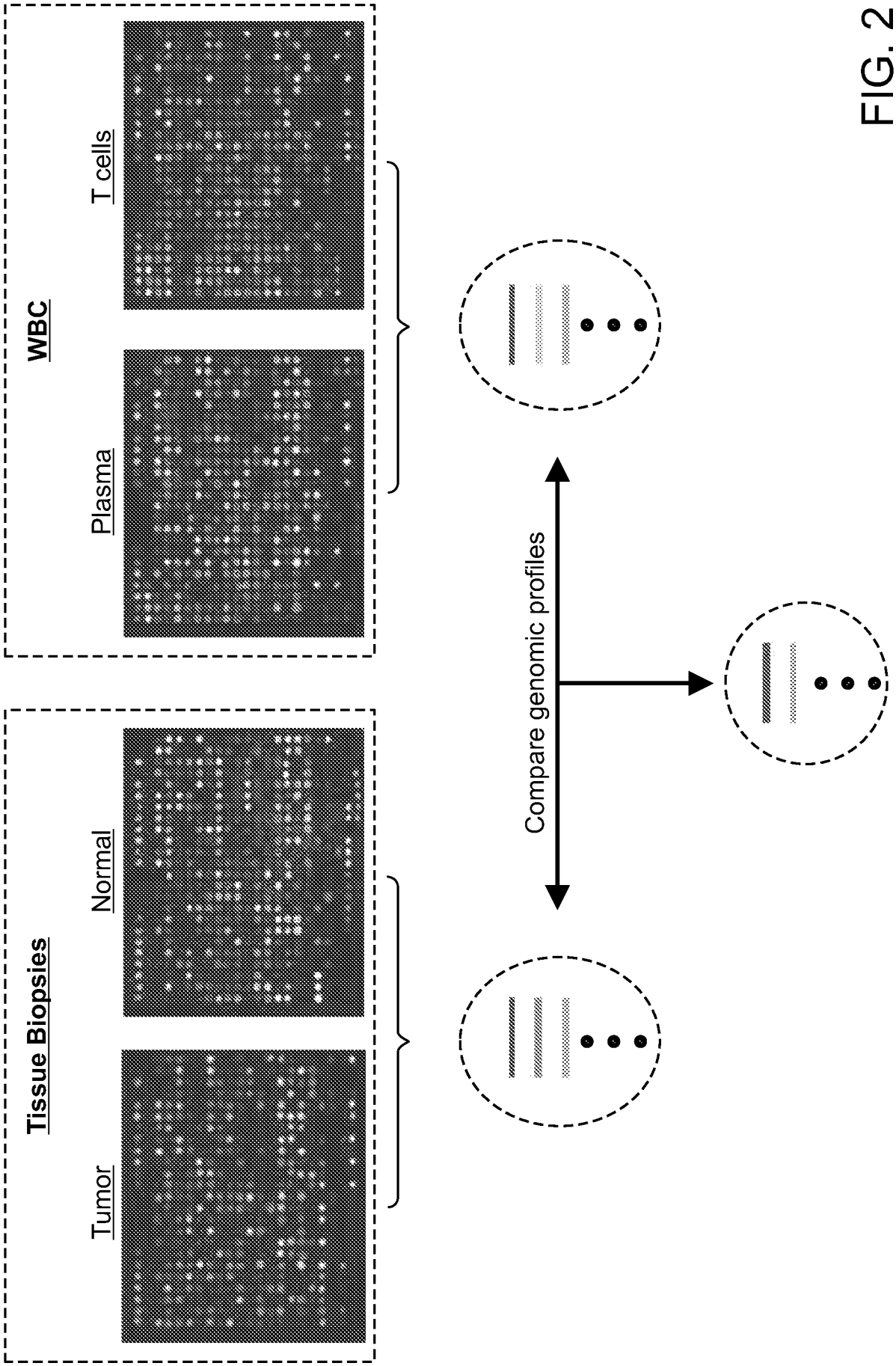
WBC
T cells
Plasma
Tissue Biopsies
Normal
Tumor
Compare genomic profiles

FIG. 2

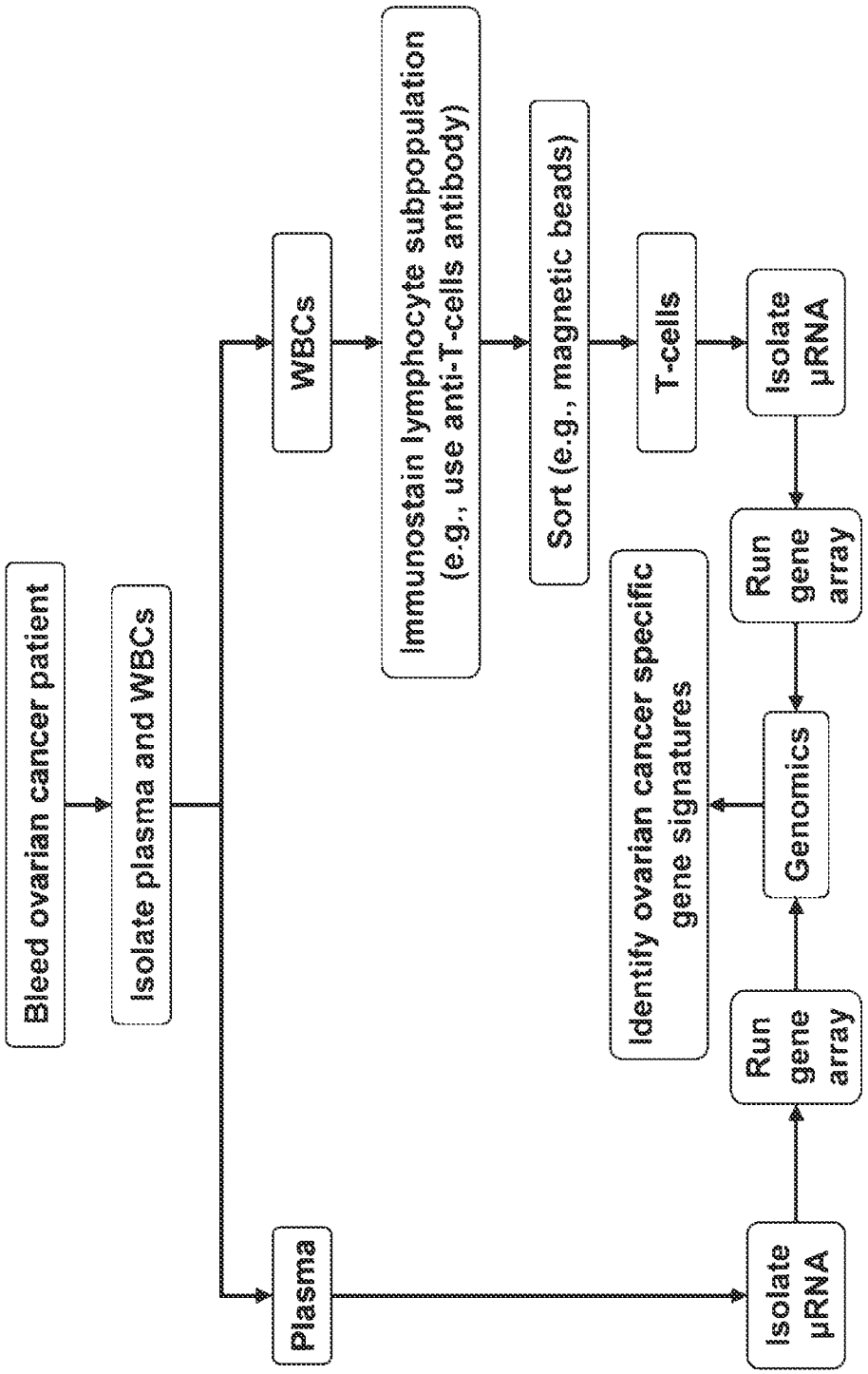
Bleed ovarian cancer patient
Isolate plasma and WBCs
WBCs
Plasma
Immunostain lymphocyte subpopulation (e.g., use anti-T-cells antibody)
Sort (e.g., magnetic beads)
T-cells
Isolate µRNA
Run gene array
Identify ovarian cancer specific gene signatures
Genomics
Run gene array
Isolate µRNA

FIG. 3

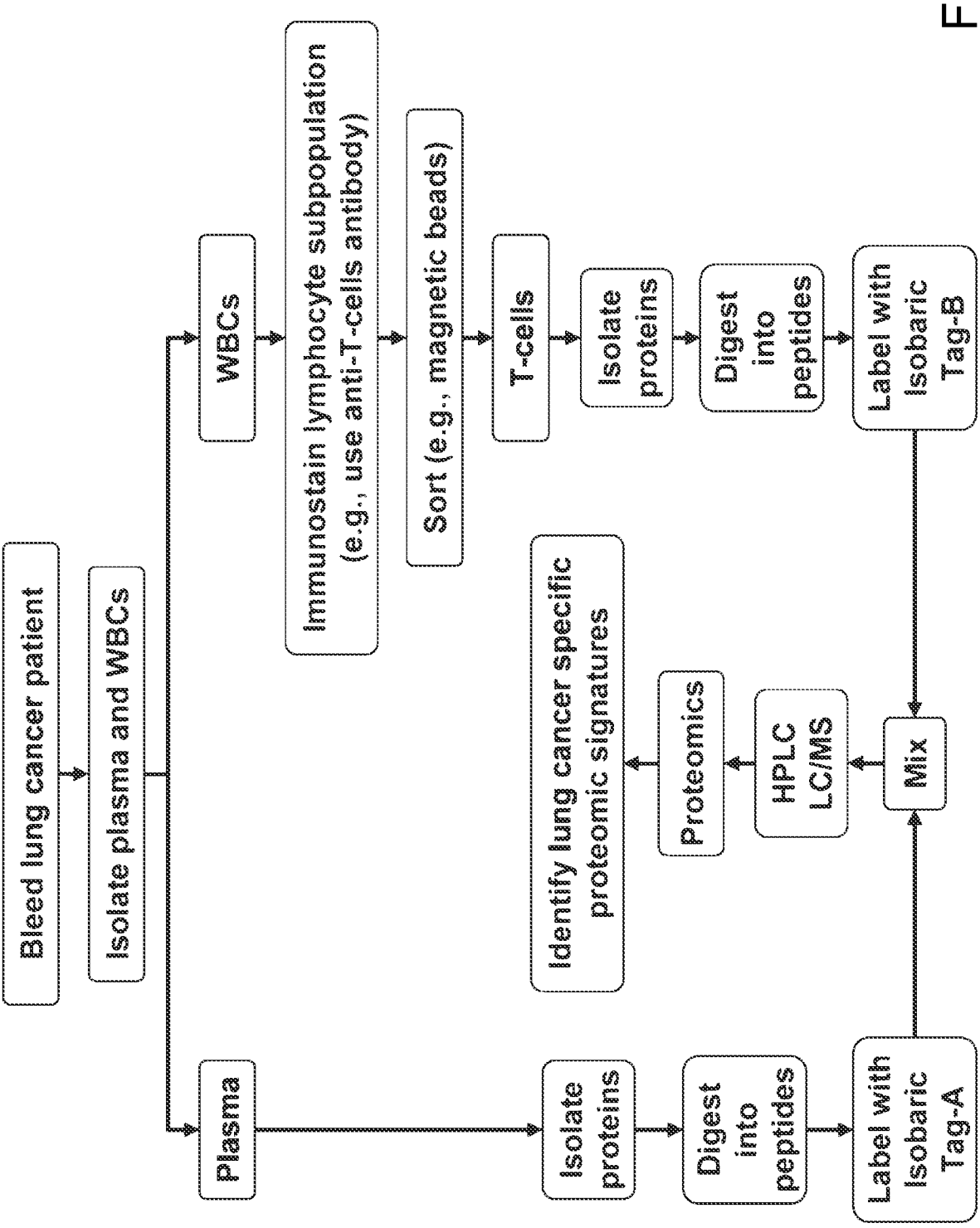
Bleed lung cancer patient
Isolate plasma and WBCs
Plasma
WBCs
Immunostain lymphocyte subpopulation (e.g., use anti-T-cells antibody)
Sort (e.g., magnetic beads)
T-cells
Isolate proteins
Digest into peptides
Label with Isobaric Tag-B
Isolate proteins
Digest into peptides
Label with Isobaric Tag-A
Mix
HPLC LC/MS
Proteomics
Identify lung cancer specific proteomic signatures

FIG. 4

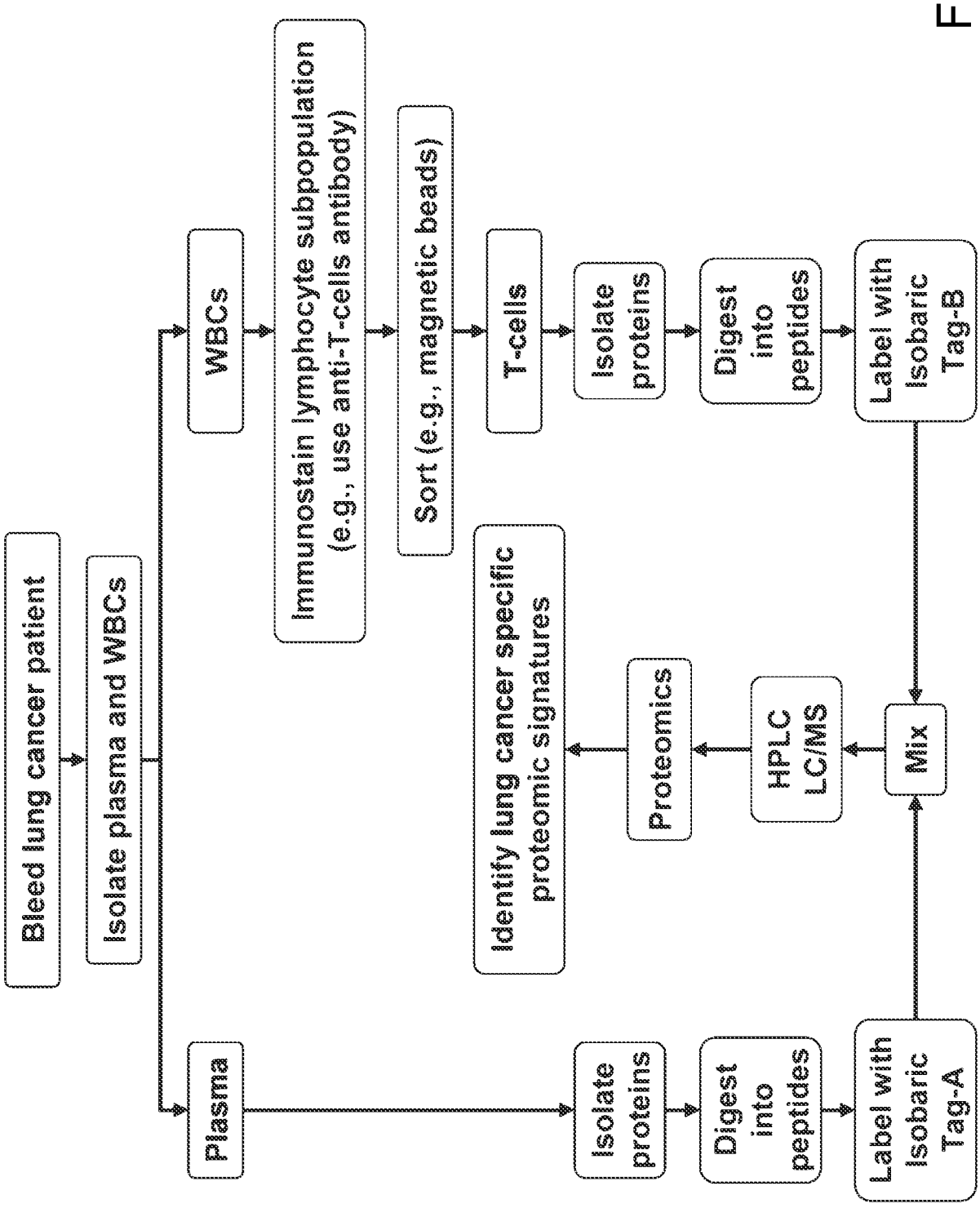
Bleed lung cancer patient
Isolate plasma and WBCs
Plasma
WBCs
Immunostain lymphocyte subpopulation (e.g., use anti-T-cells antibody)
Sort (e.g., magnetic beads)
T-cells
Isolate proteins
Digest into peptides
Label with Isobaric Tag-B
Isolate proteins
Digest into peptides
Label with Isobaric Tag-A
Identify lung cancer specific proteomic signatures
Proteomics
HPLC LC/MS
Mix

FIG. 5

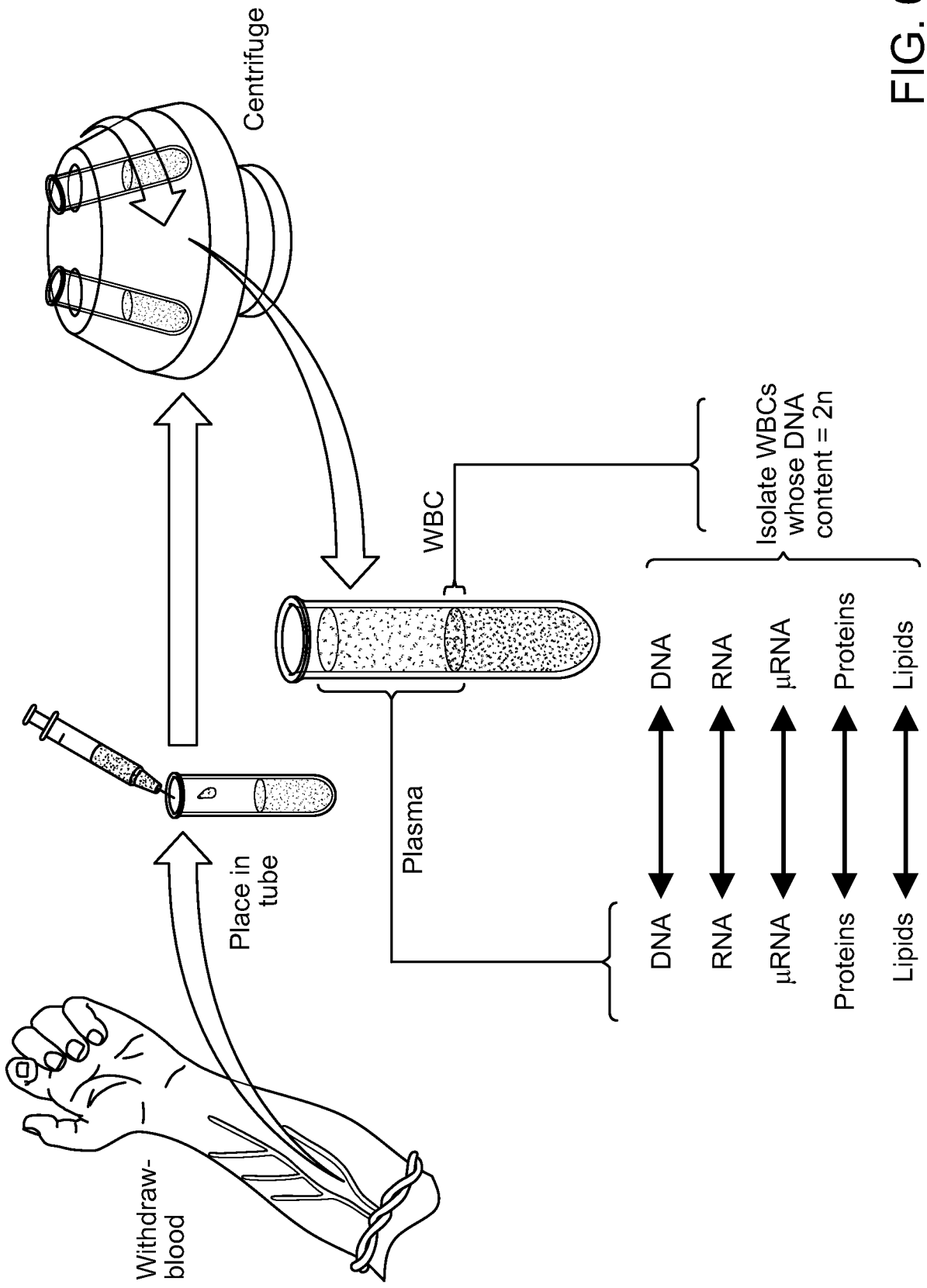
Withdraw-blood
Place in tube
Centrifuge
Plasma
WBC
Isolate WBCs whose DNA content = 2n
DNA
RNA
µRNA
Proteins
Lipids
DNA
RNA
µRNA
Proteins
Lipids

FIG. 6

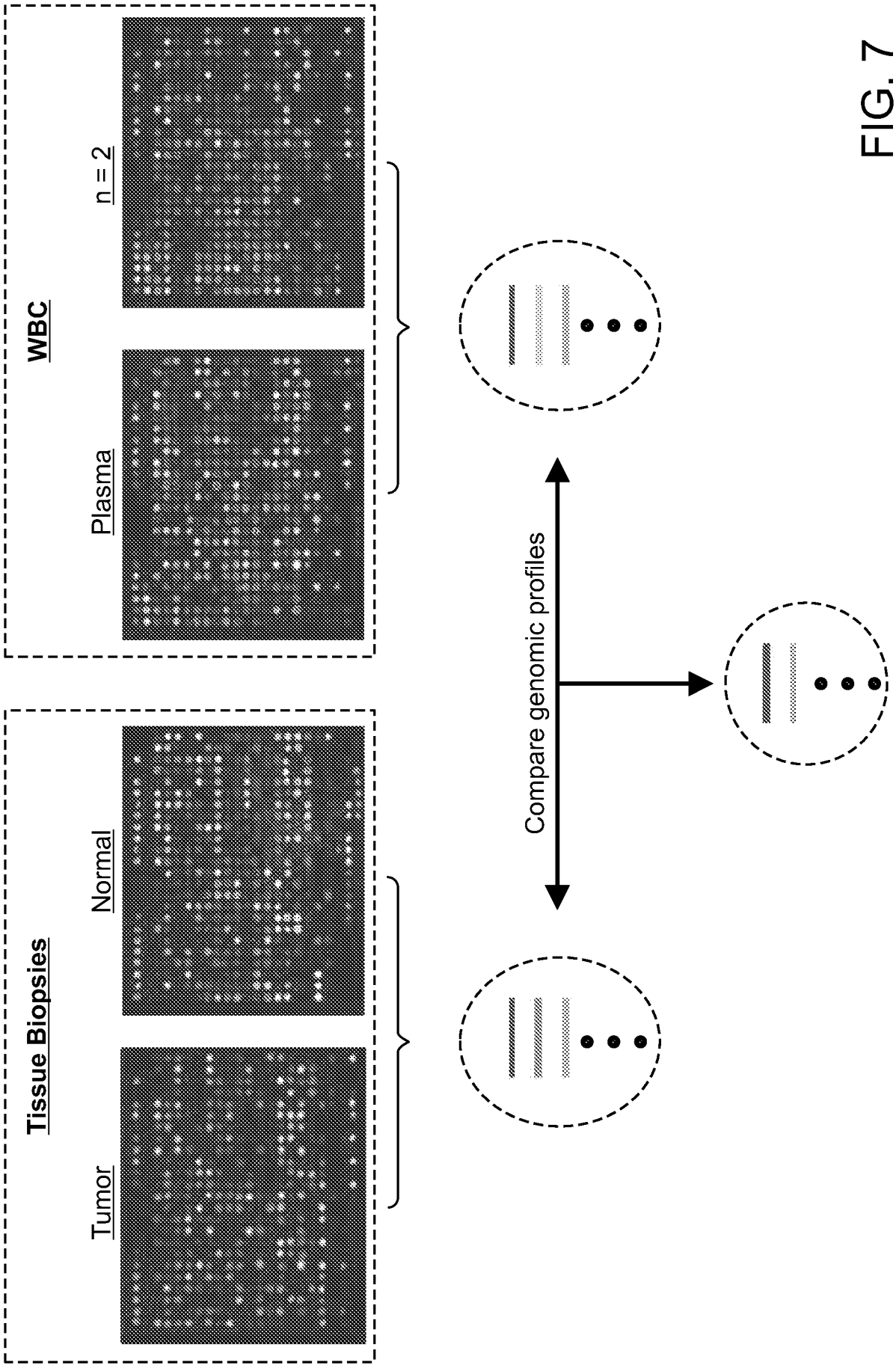
WBC
n = 2
Plasma
Tissue Biopsies
Normal
Tumor
Compare genomic profiles

FIG. 7

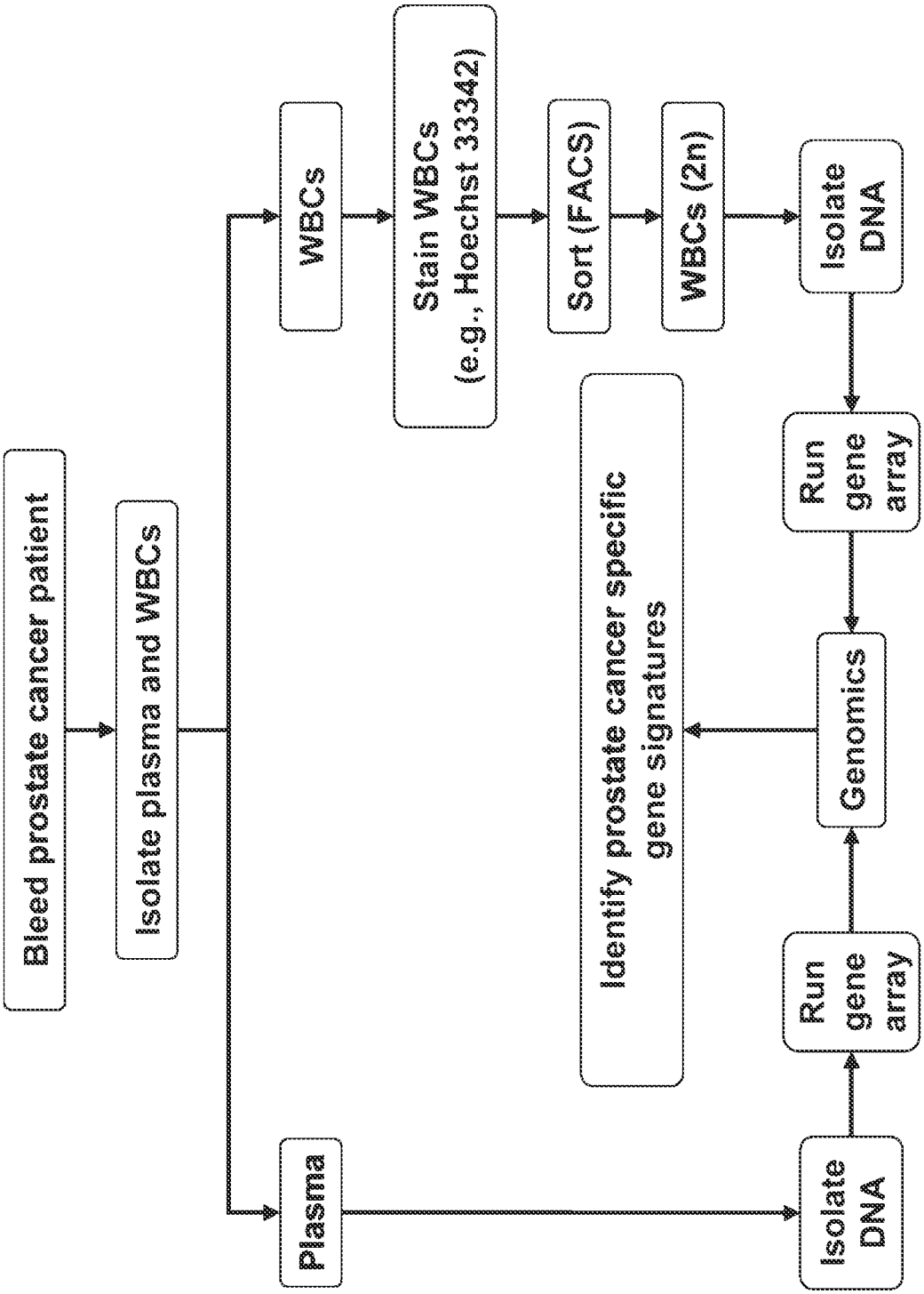
Bleed prostate cancer patient
Isolate plasma and WBCs
WBCs
Stain WBCs (e.g., Hoechst 33342)
Sort (FACS)
WBCs (2n)
Isolate DNA
Plasma
Isolate DNA
Run gene array
Run gene array
Genomics
Identify prostate cancer specific gene signatures

FIG. 8

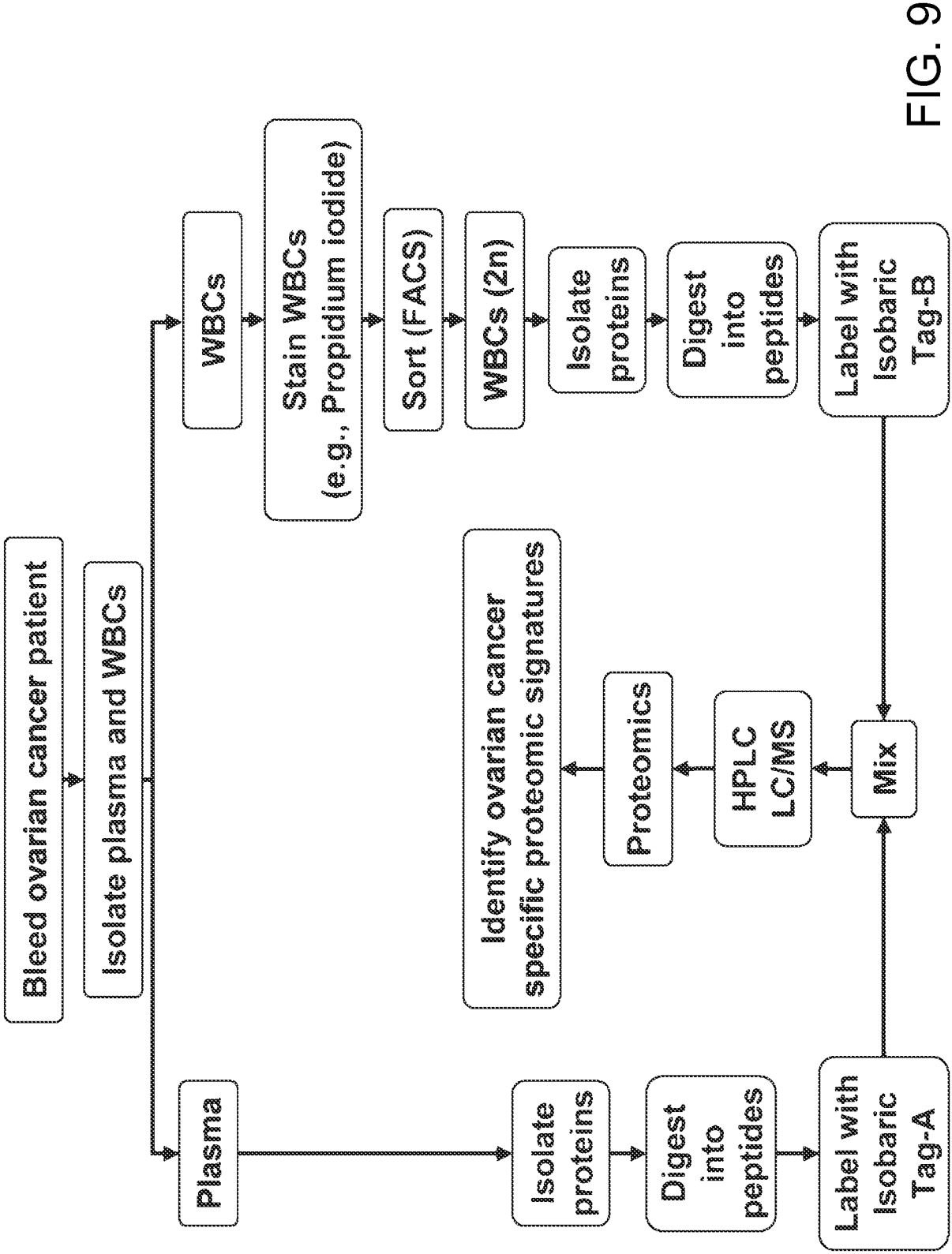
Bleed ovarian cancer patient
Isolate plasma and WBCs
Plasma
WBCs
Stain WBCs (e.g., Propidium iodide)
Sort (FACS)
WBCs (2n)
Isolate proteins
Digest into peptides
Label with Isobaric Tag-A
Label with Isobaric Tag-B
Mix
HPLC LC/MS
Proteomics
Identify ovarian cancer specific proteomic signatures

FIG. 9

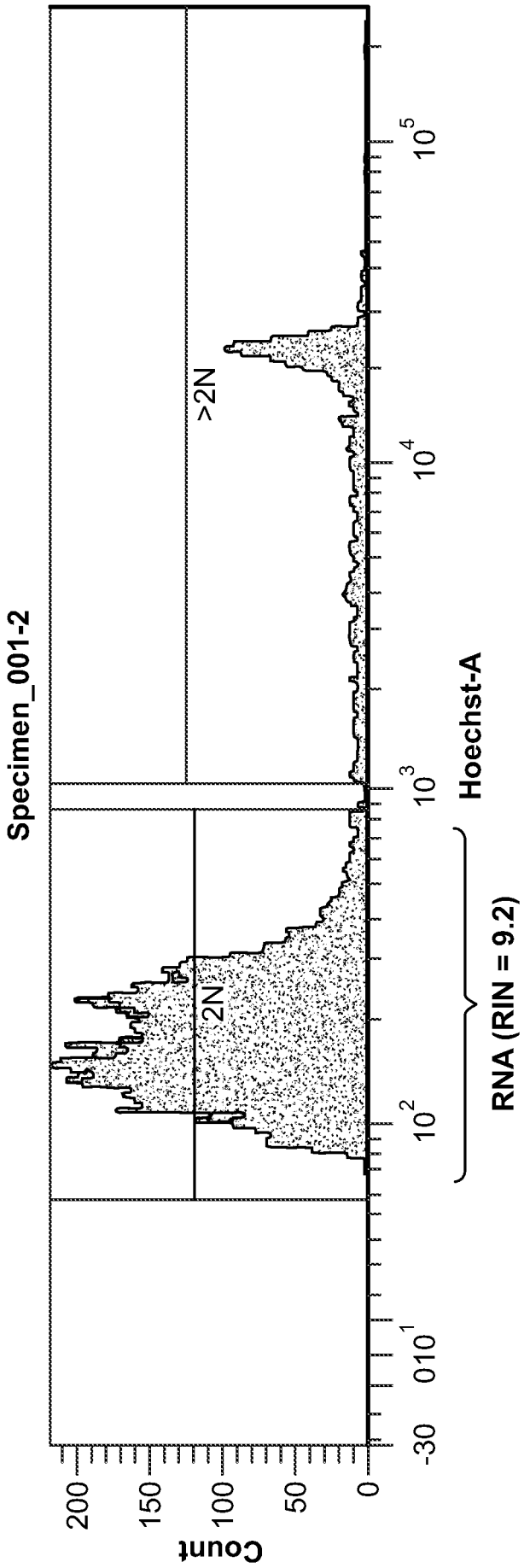
Specimen_001-2
Count
200
150
100
50
0
-30
0
10^1
10^2
10^3
10^4
10^5
Hoechst-A
RNA (RIN = 9.2)
2N
>2N

FIG. 10

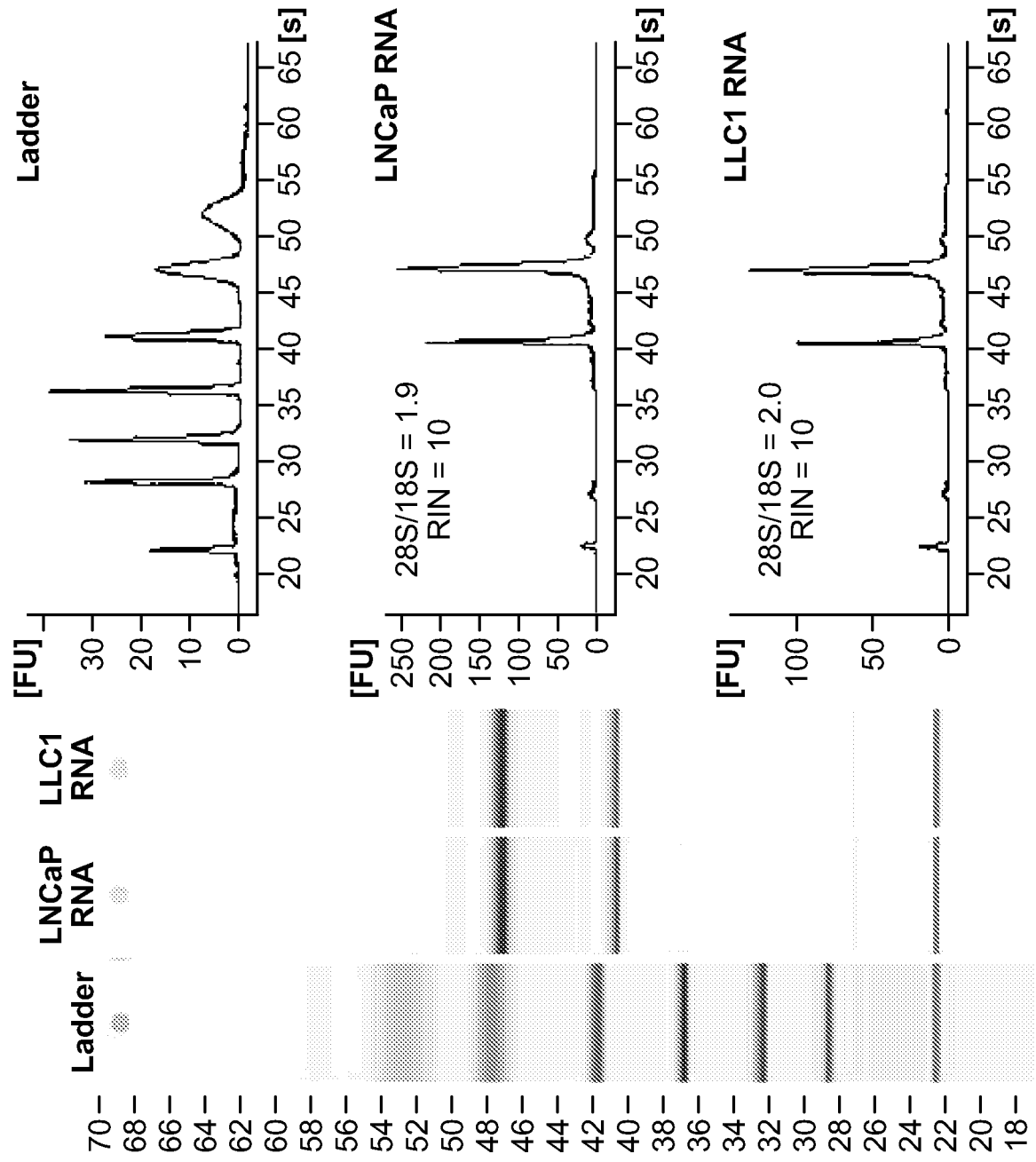
Ladder
LNCaP RNA
LLC1 RNA
[FU]
[s]
28S/18S = 1.9
RIN = 10
28S/18S = 2.0
RIN = 10

FIG. 11

Table 1. Yield and Quality of RNA Obtained from Mouse WBC

	Yield (μg)	28S/18S Ratio	RNA Integrity Number (RIN)
Macrophage*	0.53 ± 0.22	1.9 ± 0.2	9.4 ± 0.8
T cells*	0.42 ± 0.20	1.9 ± 0.3	8.4 ± 0.9
Neutrophils*	0.62 ± 0.16	1.9 ± 0.3	9.6 ± 0.7

*Mean of ≥ 6 cell preparations each.

FIG. 12

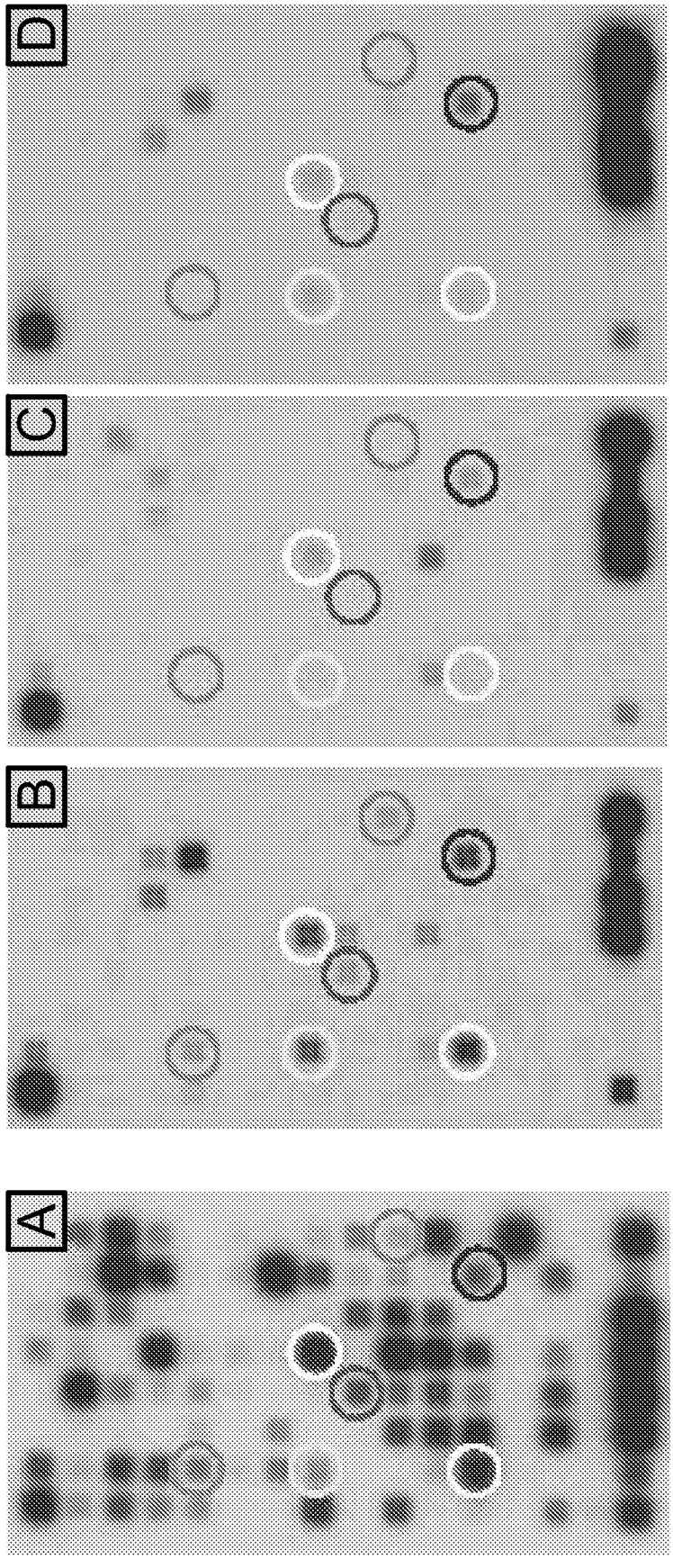

FIG. 13

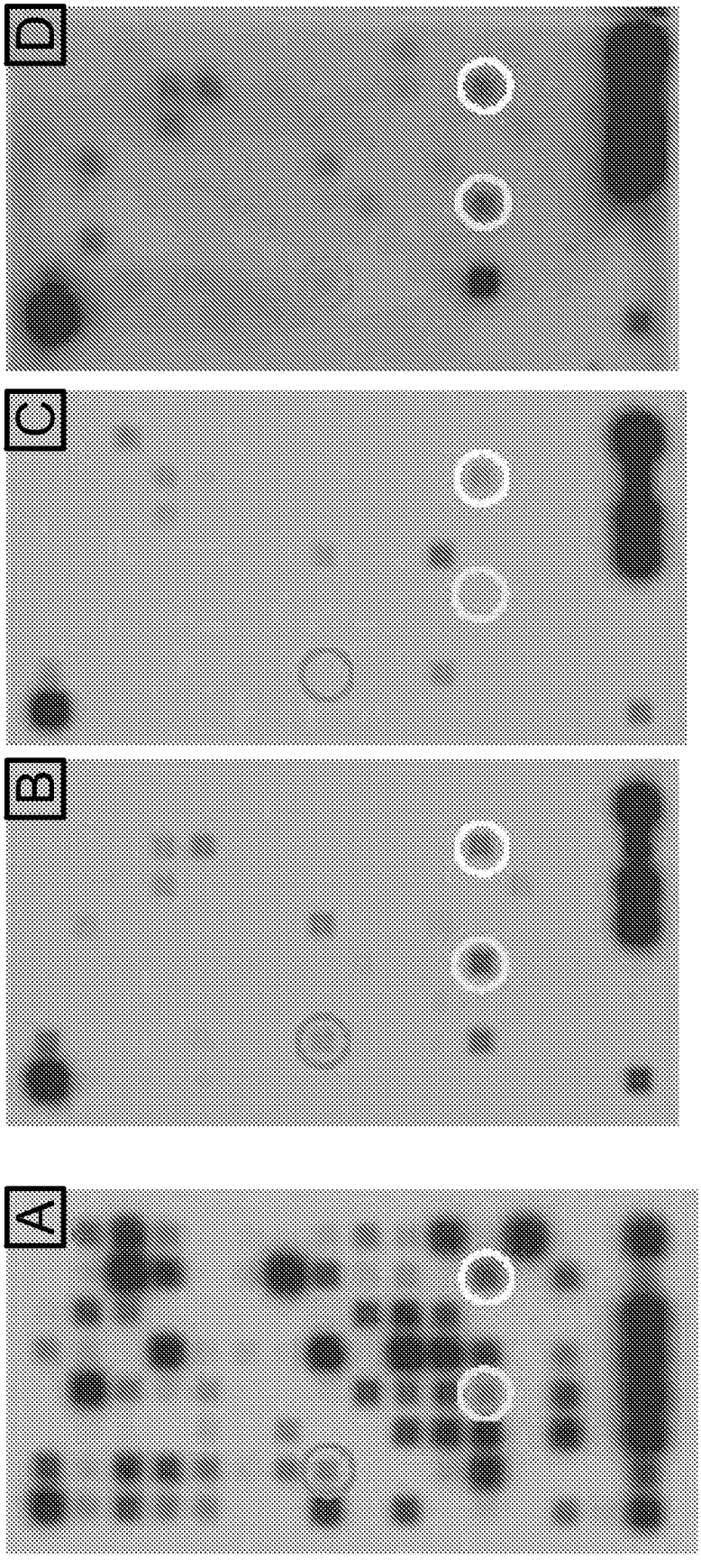

FIG. 14

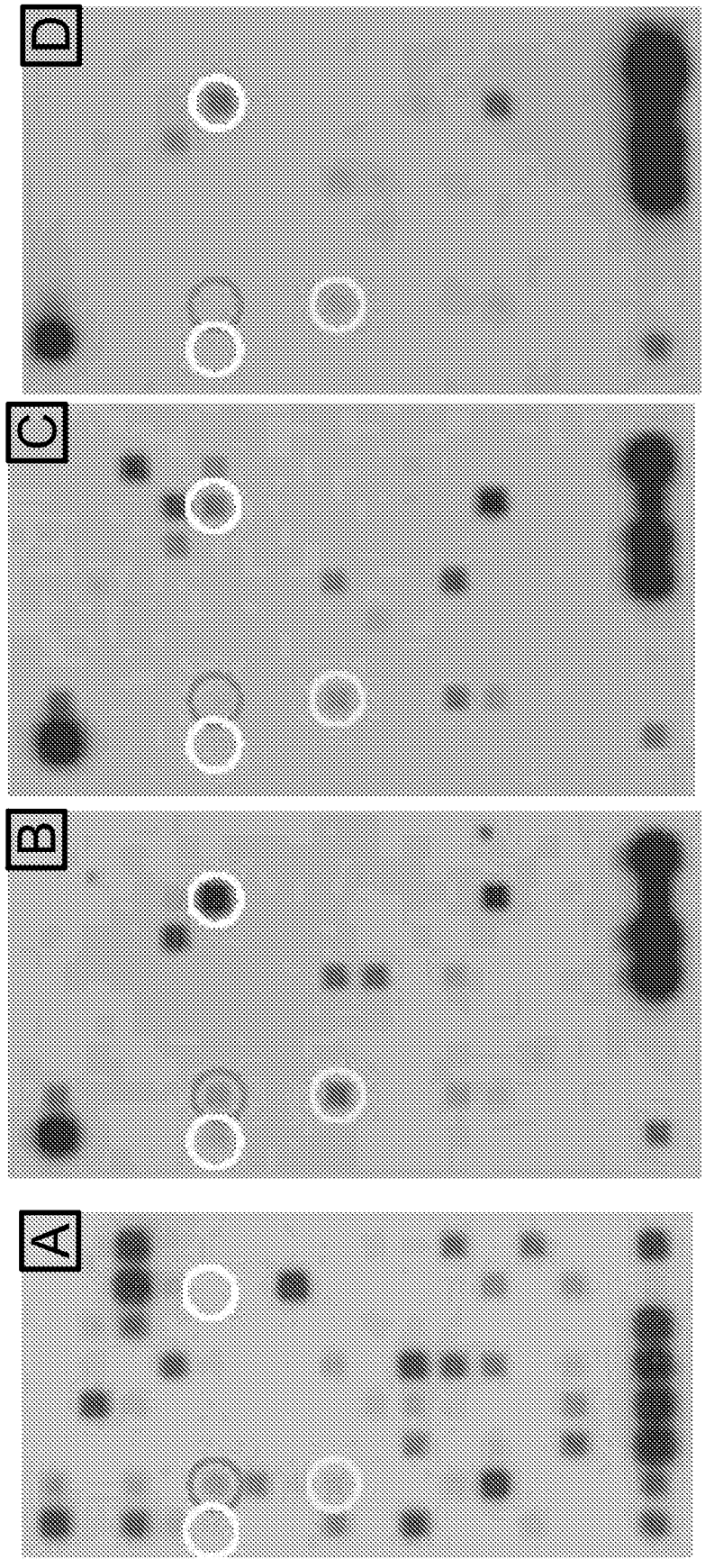

FIG. 15

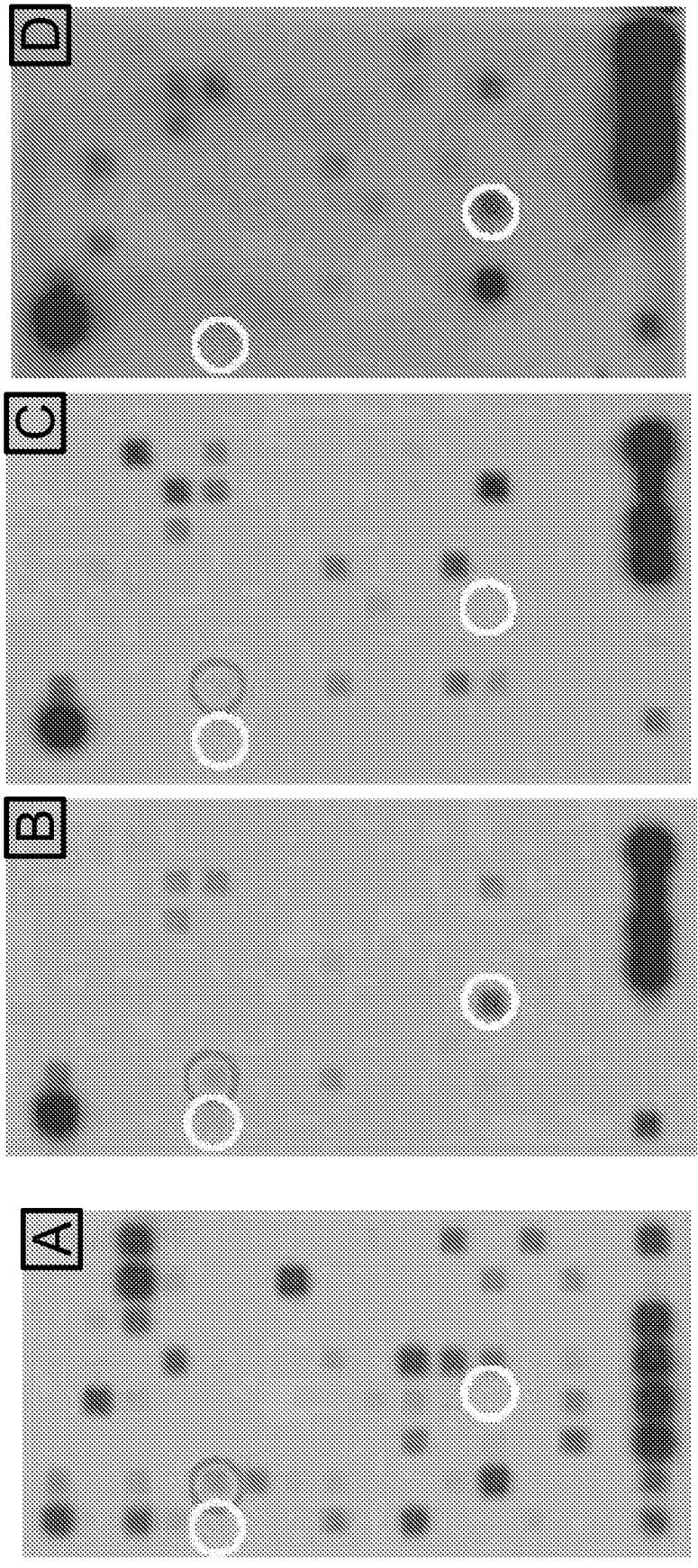

FIG. 16

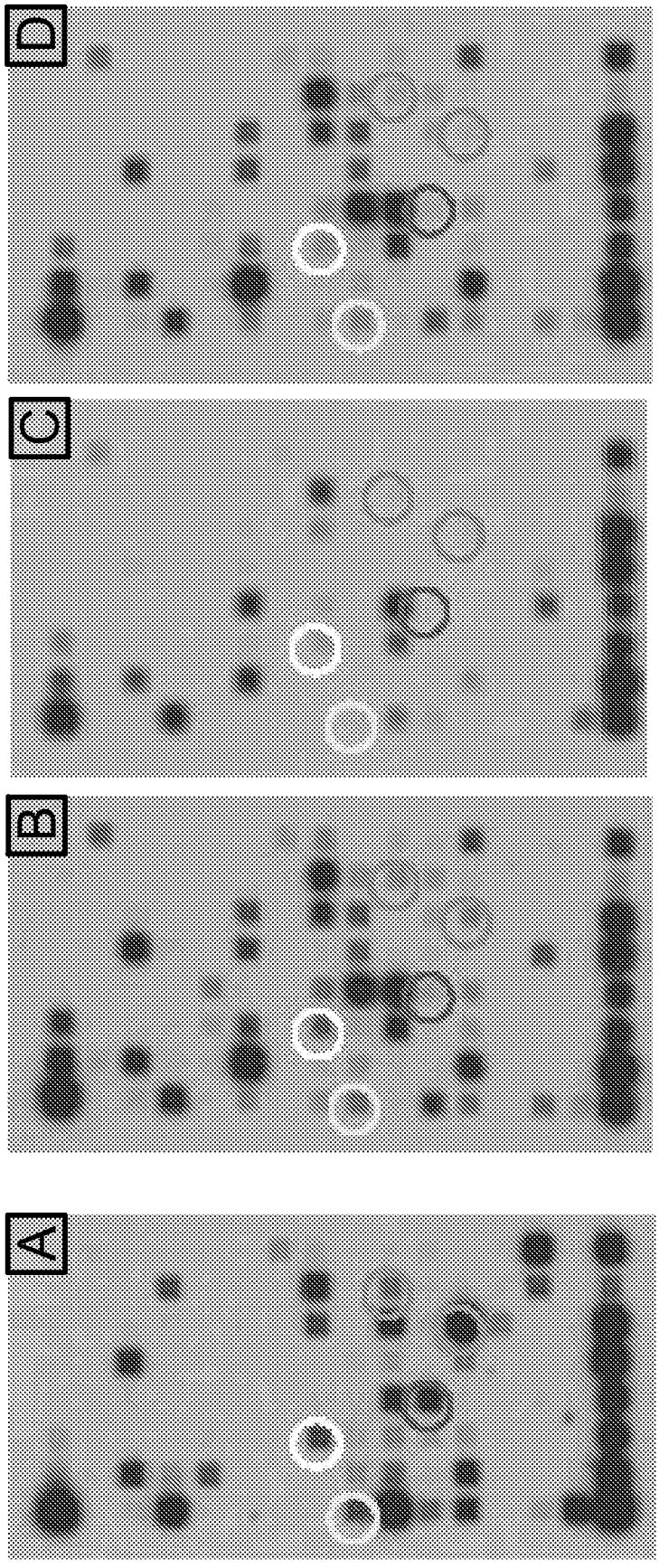

FIG. 17

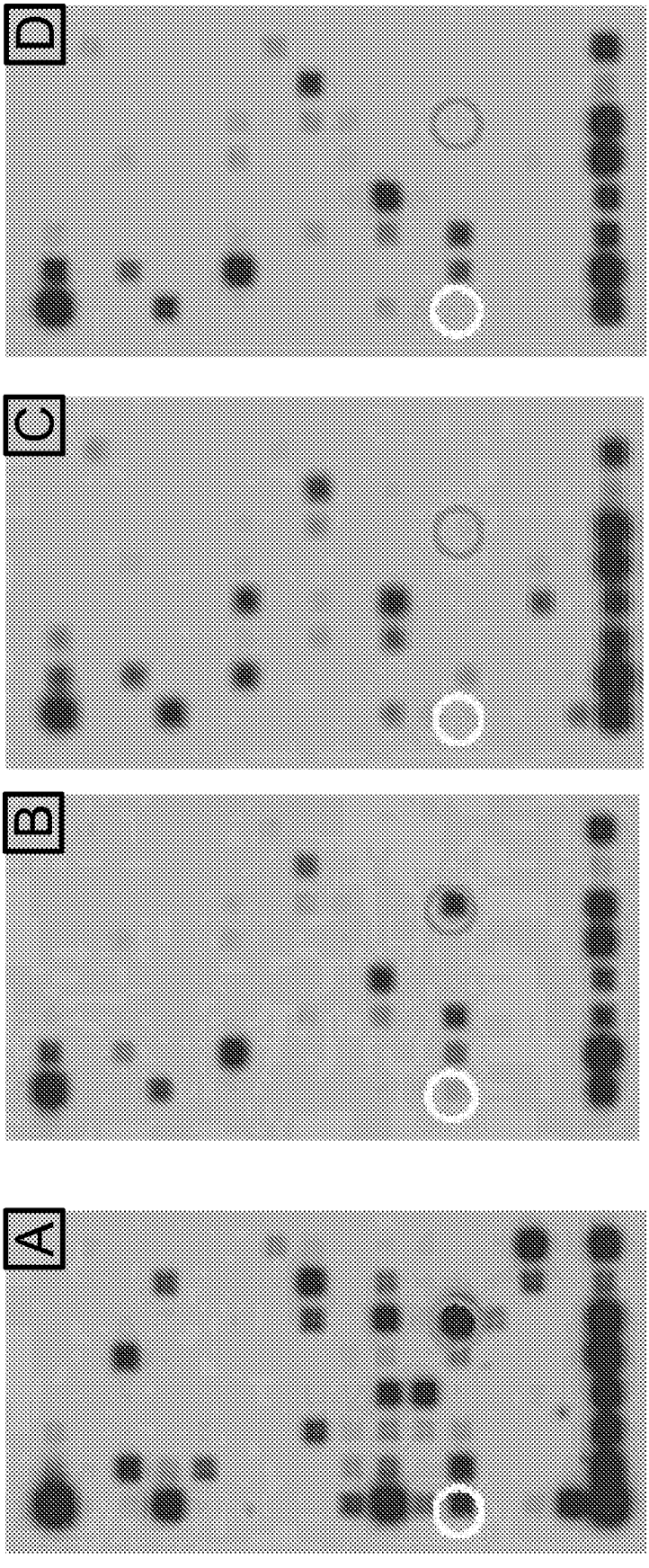

FIG. 18

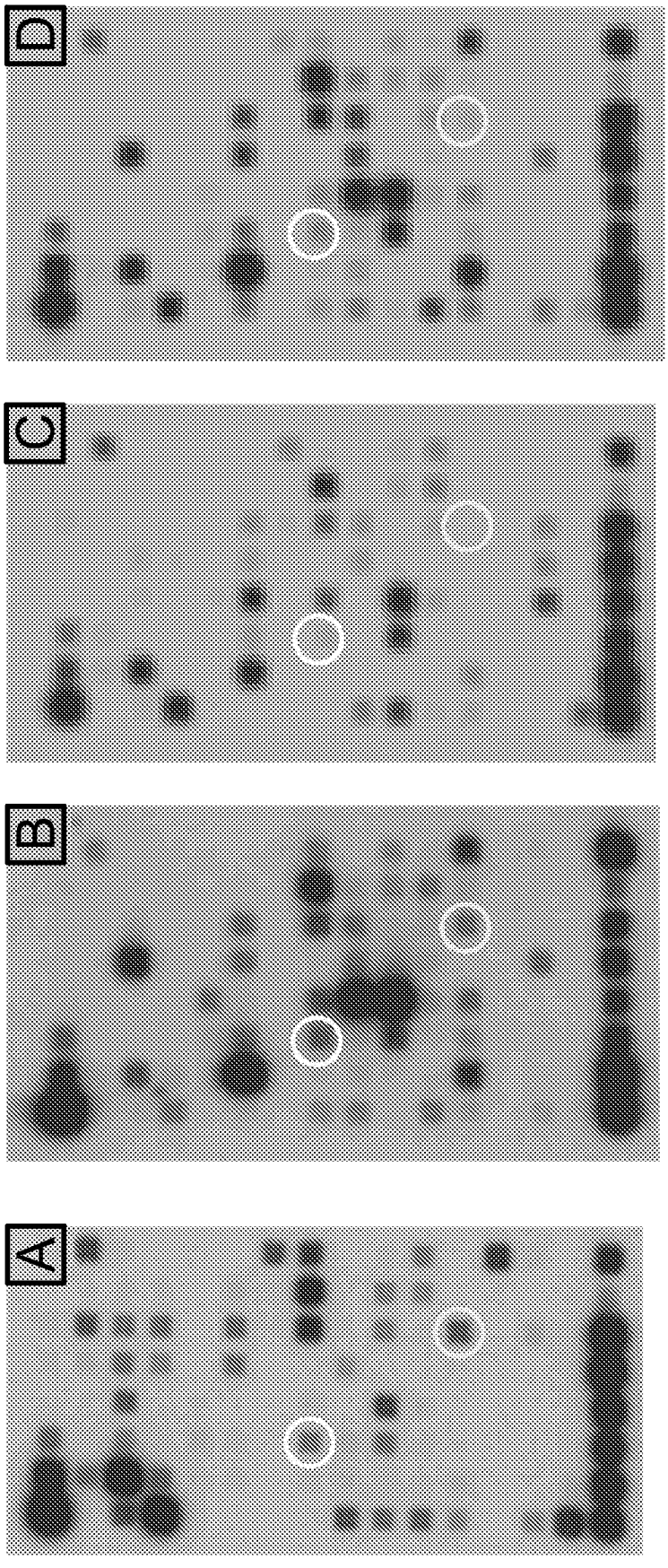

FIG. 19

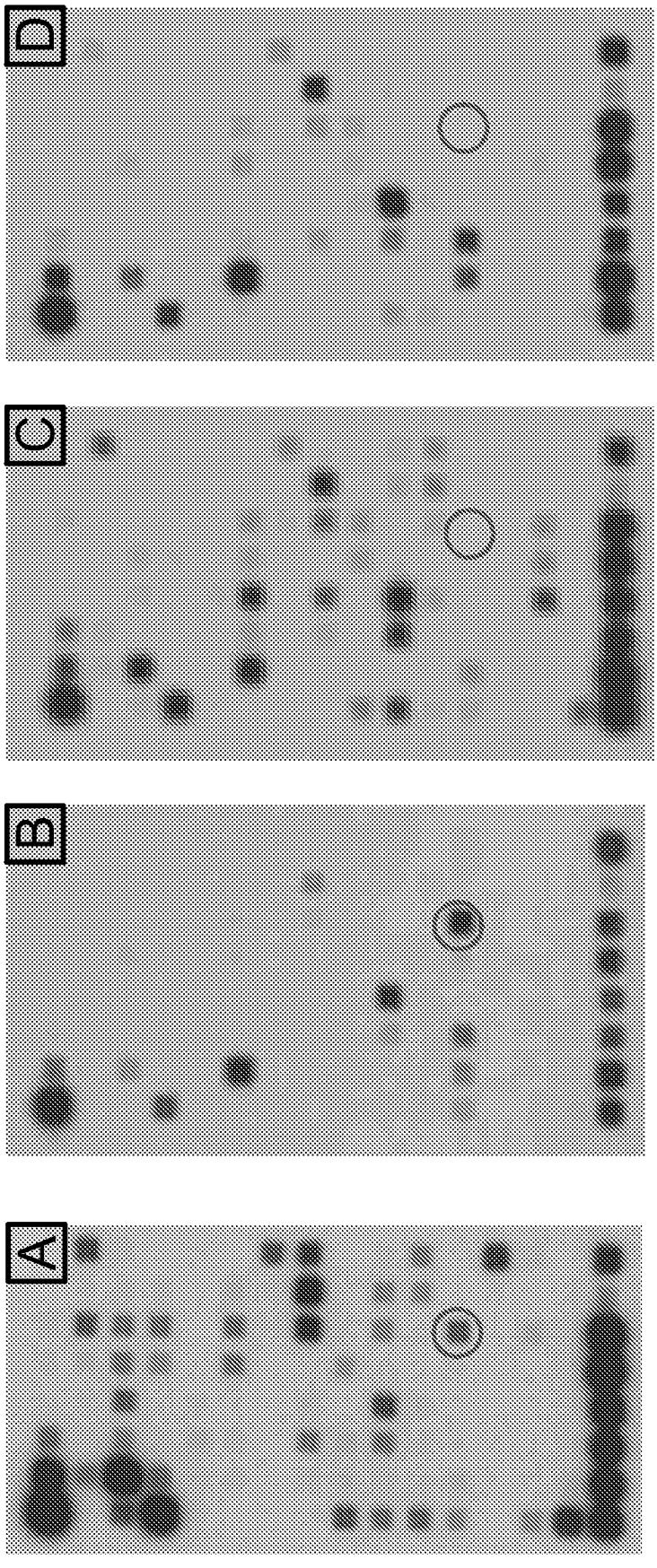

FIG. 20

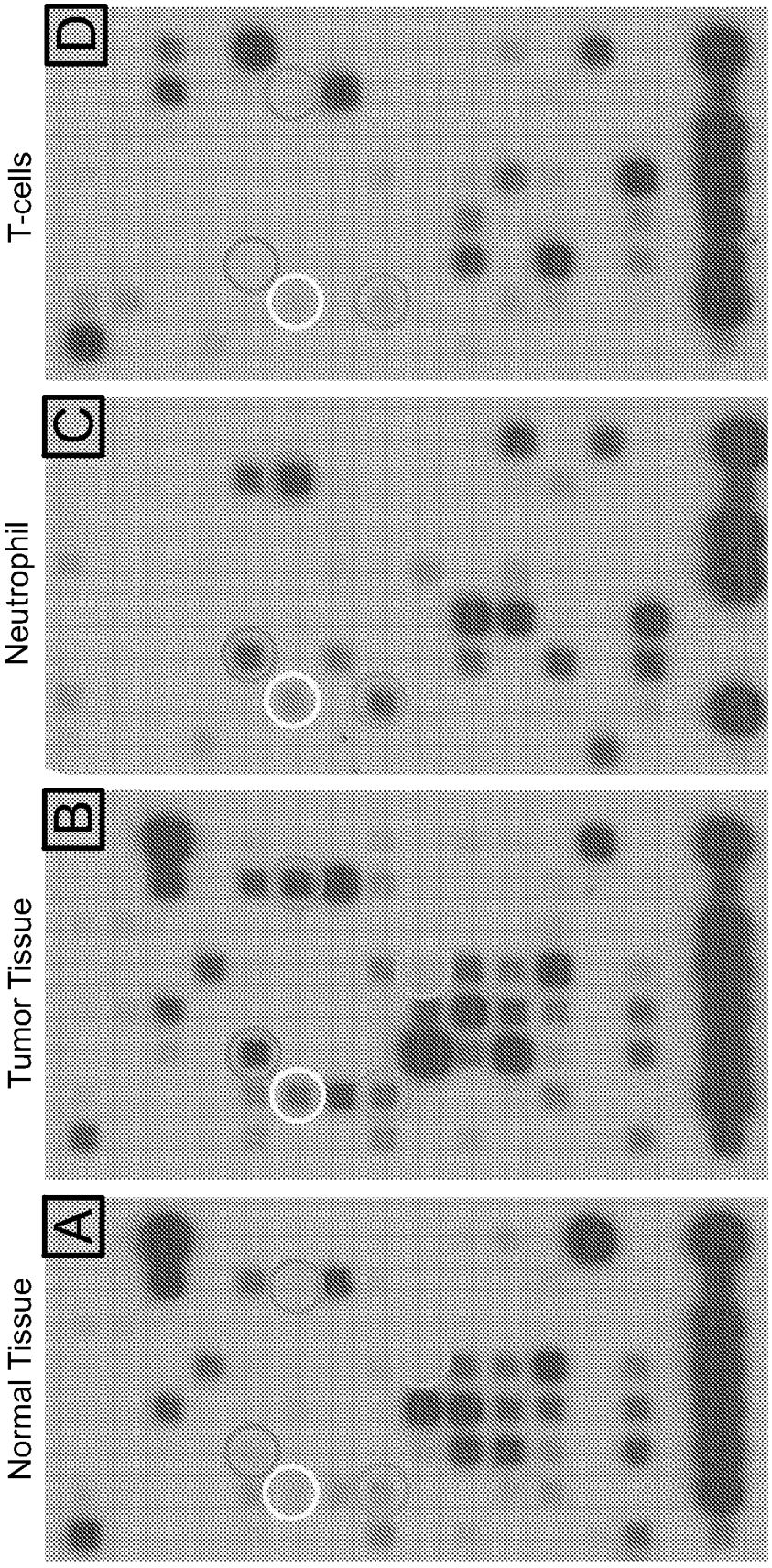

FIG. 21

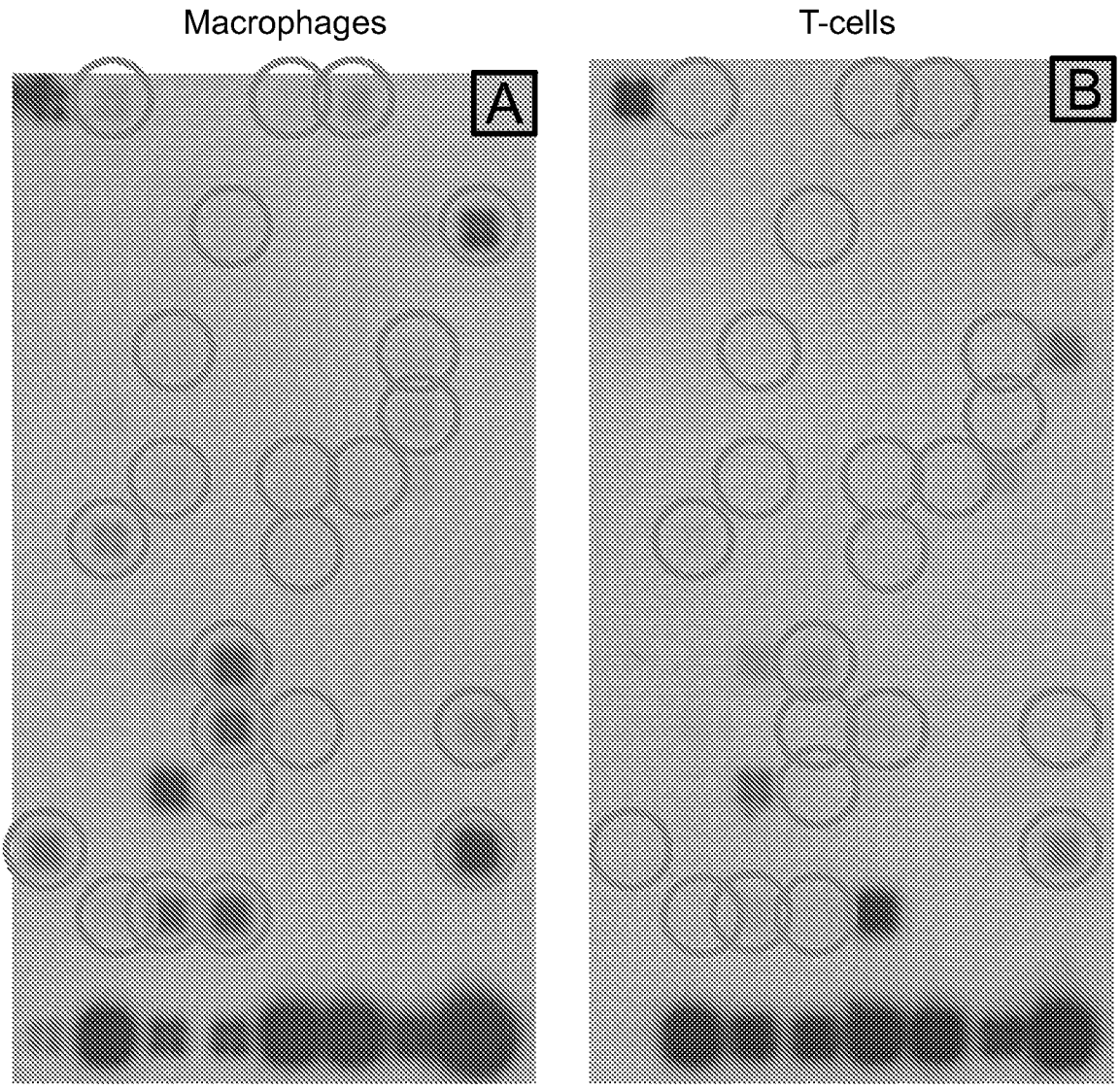
Macrophages
T-cells
A
B

FIG. 22

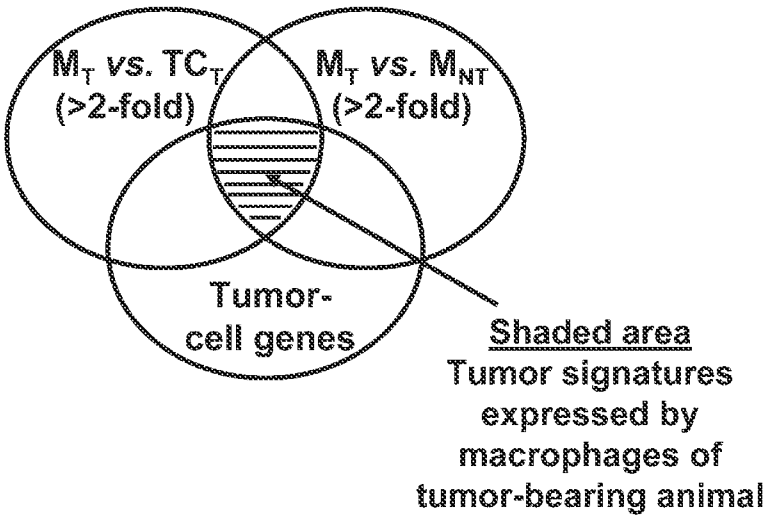
M_T *vs.* TC_T (>2-fold)
M_T *vs.* M_{NT} (>2-fold)
Tumor-cell genes
Shaded area
Tumor signatures expressed by macrophages of tumor-bearing animal

FIG. 23

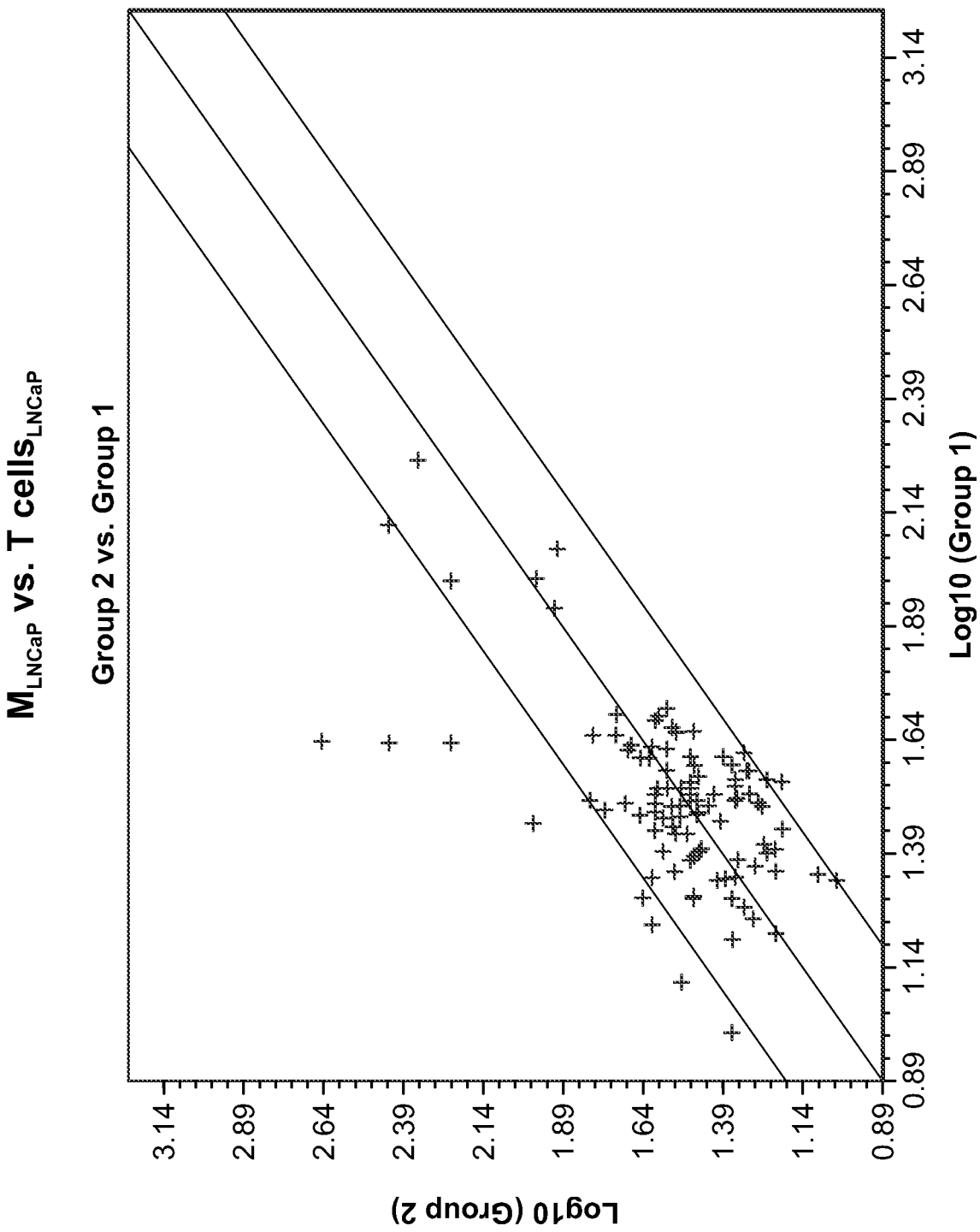
M_{LNCaP} vs. T $cells_{LNCaP}$
Group 2 vs. Group 1
Log10 (Group 2)
Log10 (Group 1)
3.14
2.89
2.64
2.39
2.14
1.89
1.64
1.39
1.14
0.89

FIG. 24A

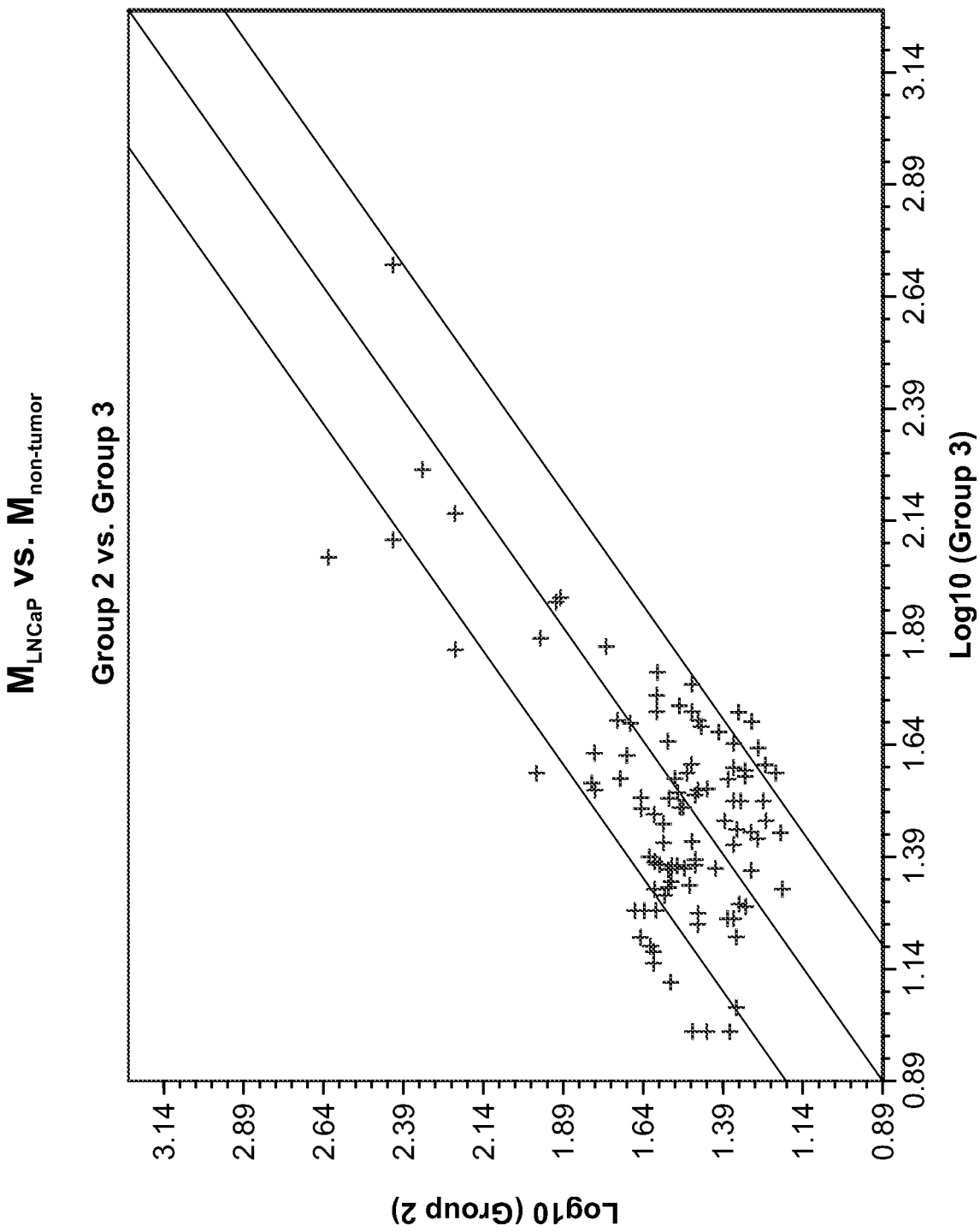
M_{LNCaP} vs. $M_{non-tumor}$
Group 2 vs. Group 3
Log10 (Group 2)
Log10 (Group 3)
0.89
1.14
1.39
1.64
1.89
2.14
2.39
2.64
2.89
3.14

FIG. 24B

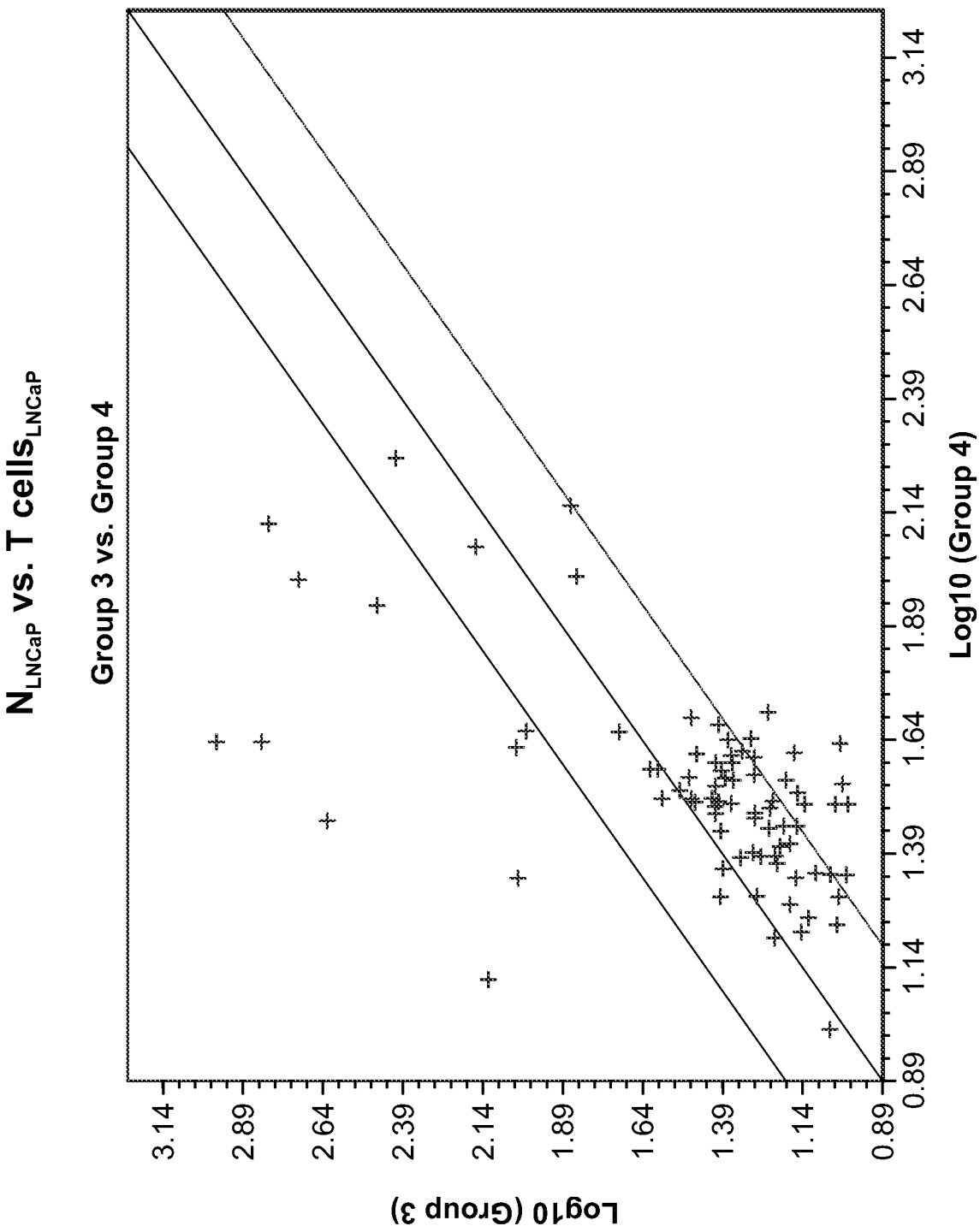
N_{LNCaP} vs. T $cells_{LNCaP}$
Group 3 vs. Group 4
Log10 (Group 3)
Log10 (Group 4)
3.14
2.89
2.64
2.39
2.14
1.89
1.64
1.39
1.14
0.89

FIG. 24C

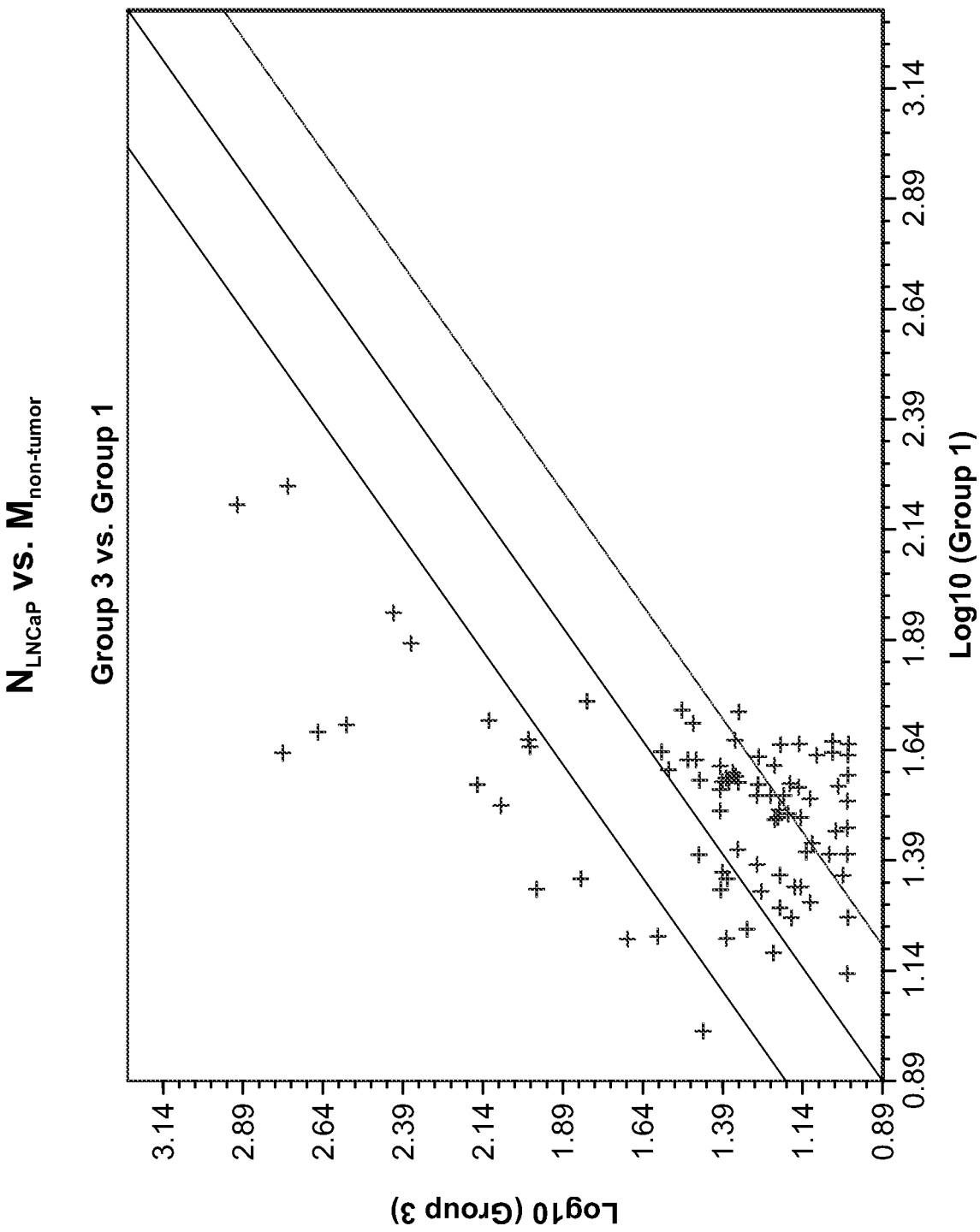
N_{LNCaP} vs. $M_{non-tumor}$
Group 3 vs. Group 1
Log10 (Group 3)
Log10 (Group 1)
0.89
1.14
1.39
1.64
1.89
2.14
2.39
2.64
2.89
3.14

FIG. 24D

Table 5. Expression of cancer-related genes within phagocytic neutrophils (N) and macrophages (M)

	Over-expressed Genes	**LNCaP** (human prostate Ca.) (nude mice)		**LS174T** (human colon Ca.) (nude mice)		**B16F10** (mouse melanoma) (black mice)		**LLC1** (mouse lung Ca.) (black mice)	
		M	N	M	N	M	N	M	N
	BAK1	-	-	-	-	+	+	-	-
	EGFR	-	-	+	+	-	-	-	-
	ERBB2	-	+	+	+	-	-	-	-
	FOS	-	-	-	+	-	-	-	-
	JUN	+	+	-	+	-	-	-	+
	MAP2K1	-	+	-	-	-	-	-	-
	Mdm2								+
	MMP2	-	+	-	-	-	-	-	-
	PDGFB	-	+	-	-	-	-	-	+
	Plaur								+
	RB1	-	+	-	-	-	-	+	-
	SNCG	+	+	-	-	-	-	-	-
	SERPINB2	+	-	+	-	-	-	-	-
	SPP1	-	-	-	-	-	+	+	+
Total	12	3	7	3	4	1	2	2	5
Gene overexpressed in phagocytes (M+N):		8		5		2		6	

Red = oncogenes; blue = tumor-specific genes; and black = tumor-related genes.

☐ = gene acquired/expressed simultaneously by M and N

FIG. 25

Table 3. Upregulated (>2-fold) cancer-related genes in macrophages of ovarian cancer patient

Gene Symbol	Macrophages/T cells
AKT1*	**4.62**
APAF1	4.95
ATM	5.26
CDC25A	2.01
CDKN1A	4.57
ETS2	**3.47**
FOS	**6.49**
IL8	3.62
ITGA4	6.58
ITGA6	3.53
ITGAV	2.50
JUN	**3.01**
MAP2K1	3.09
NFKBIA	2.77
PLAU	2.13
PLAUR	38.79
RAF1	**8.13**
SERPINB2	5.37
SYK	20.81
TIMP1	2.04
TNF	3.75
TNFRSF10B	3.67
TNFRSF1A	6.96

*** = oncogene**

FIG. 26

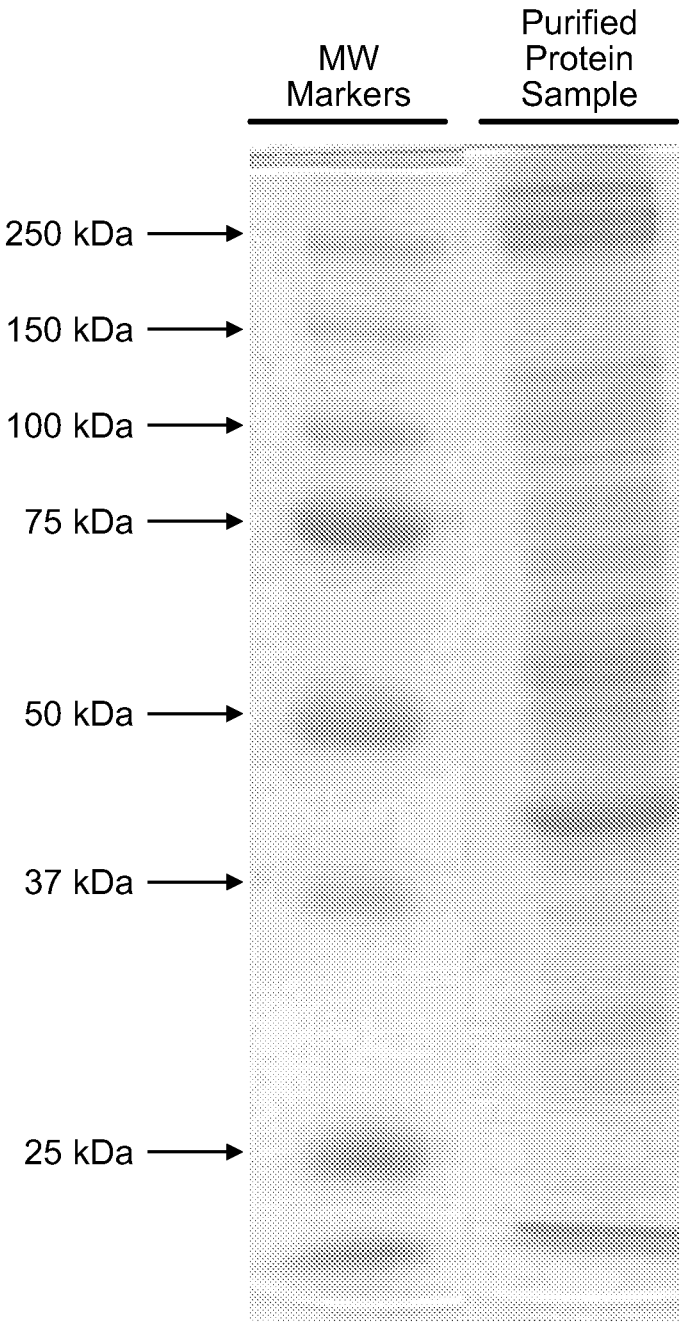
MW Markers
Purified Protein Sample
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa

FIG. 27

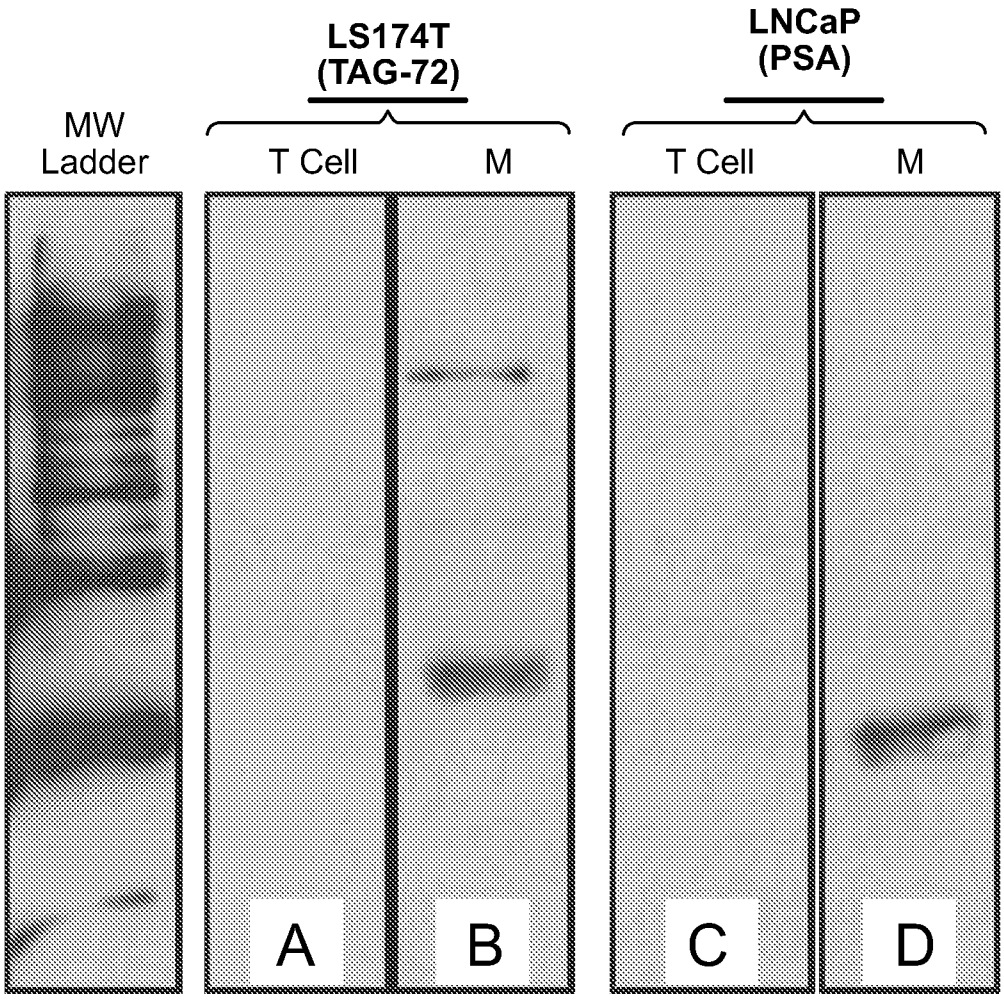

FIG. 28