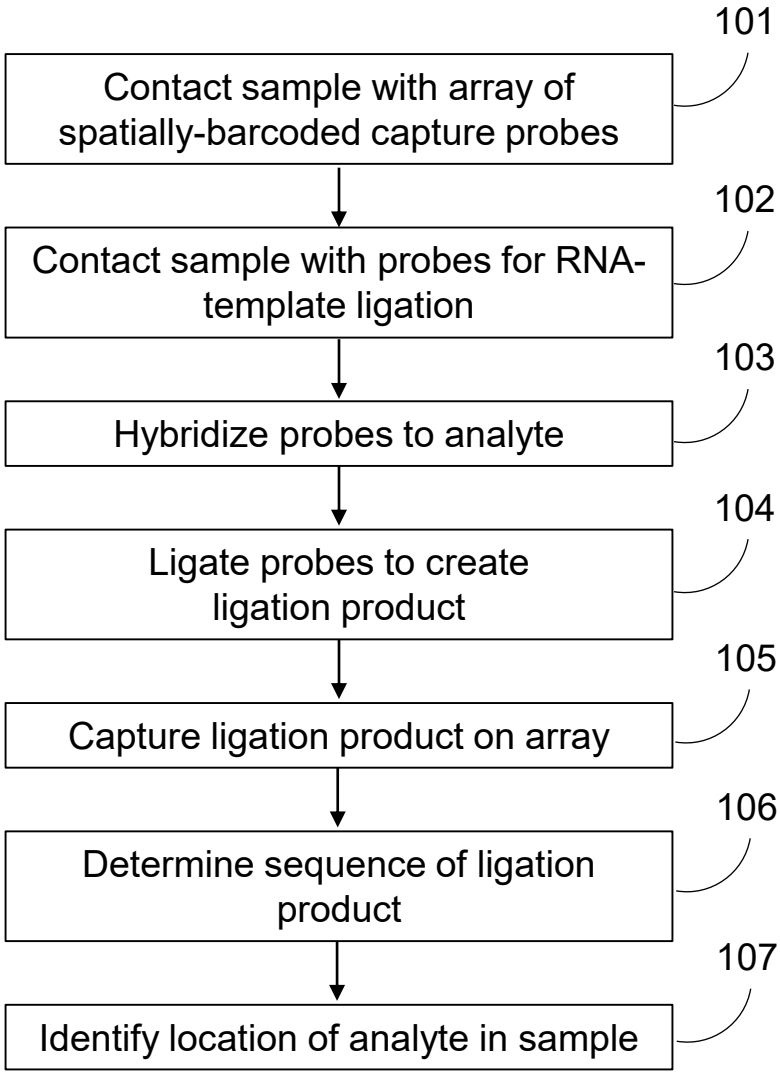

101
Contact sample with array of spatially-barcoded capture probes
102
Contact sample with probes for RNA-template ligation
103
Hybridize probes to analyte
104
Ligate probes to create ligation product
105
Capture ligation product on array
106
Determine sequence of ligation product
107
Identify location of analyte in sample

FIG. 1

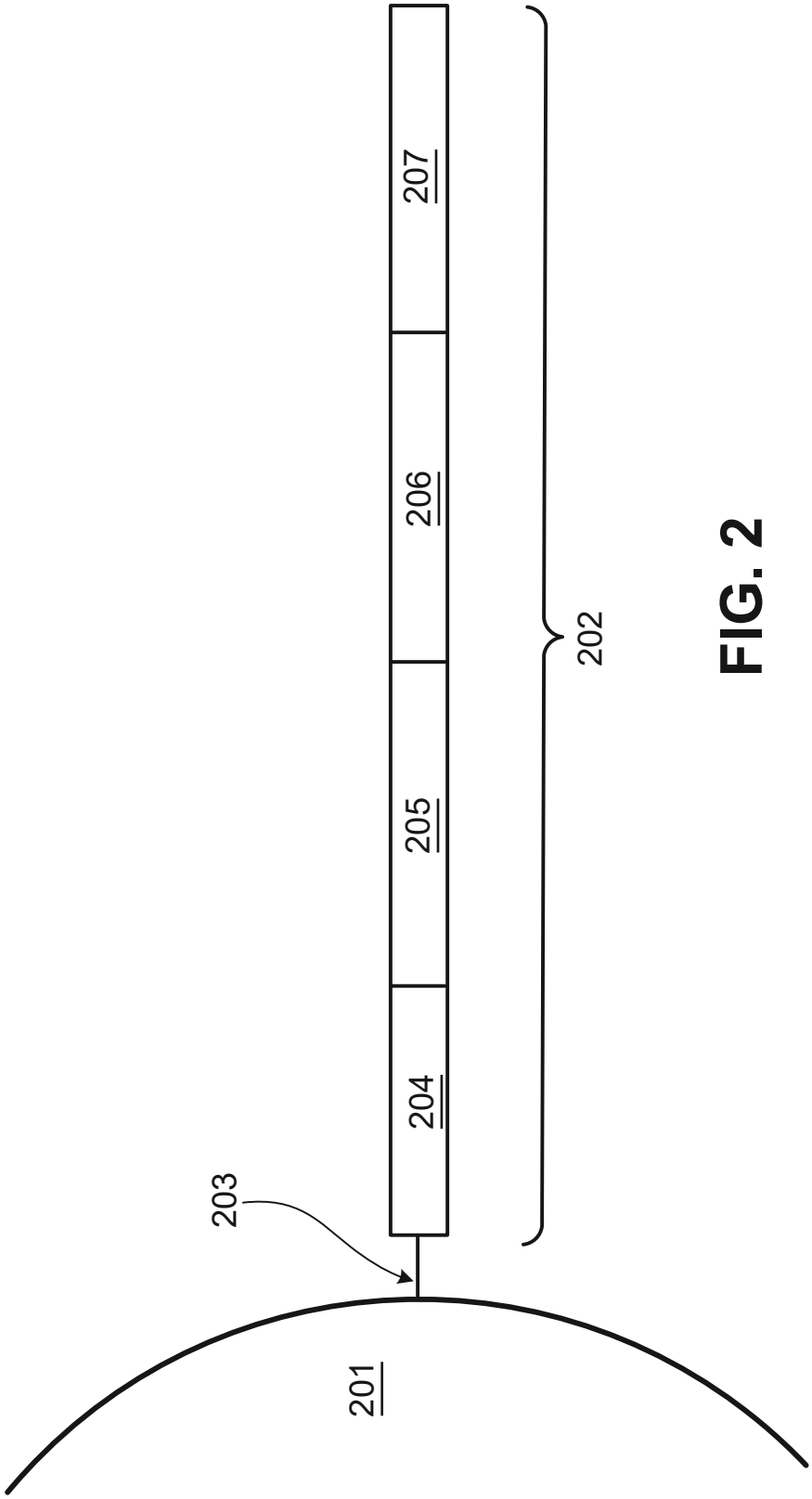


FIG. 2

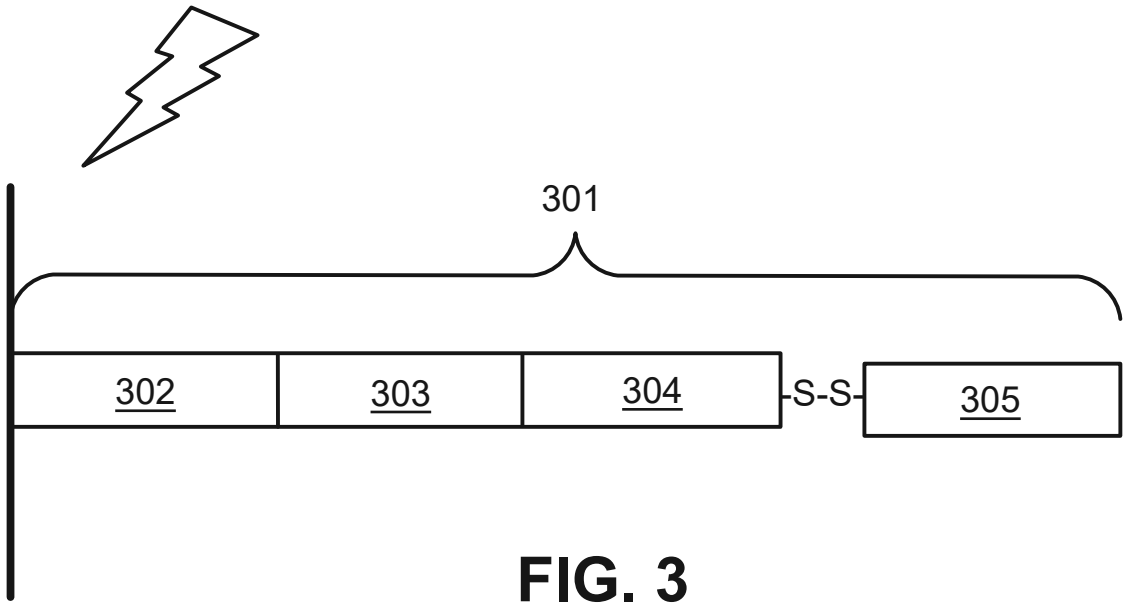


FIG. 3

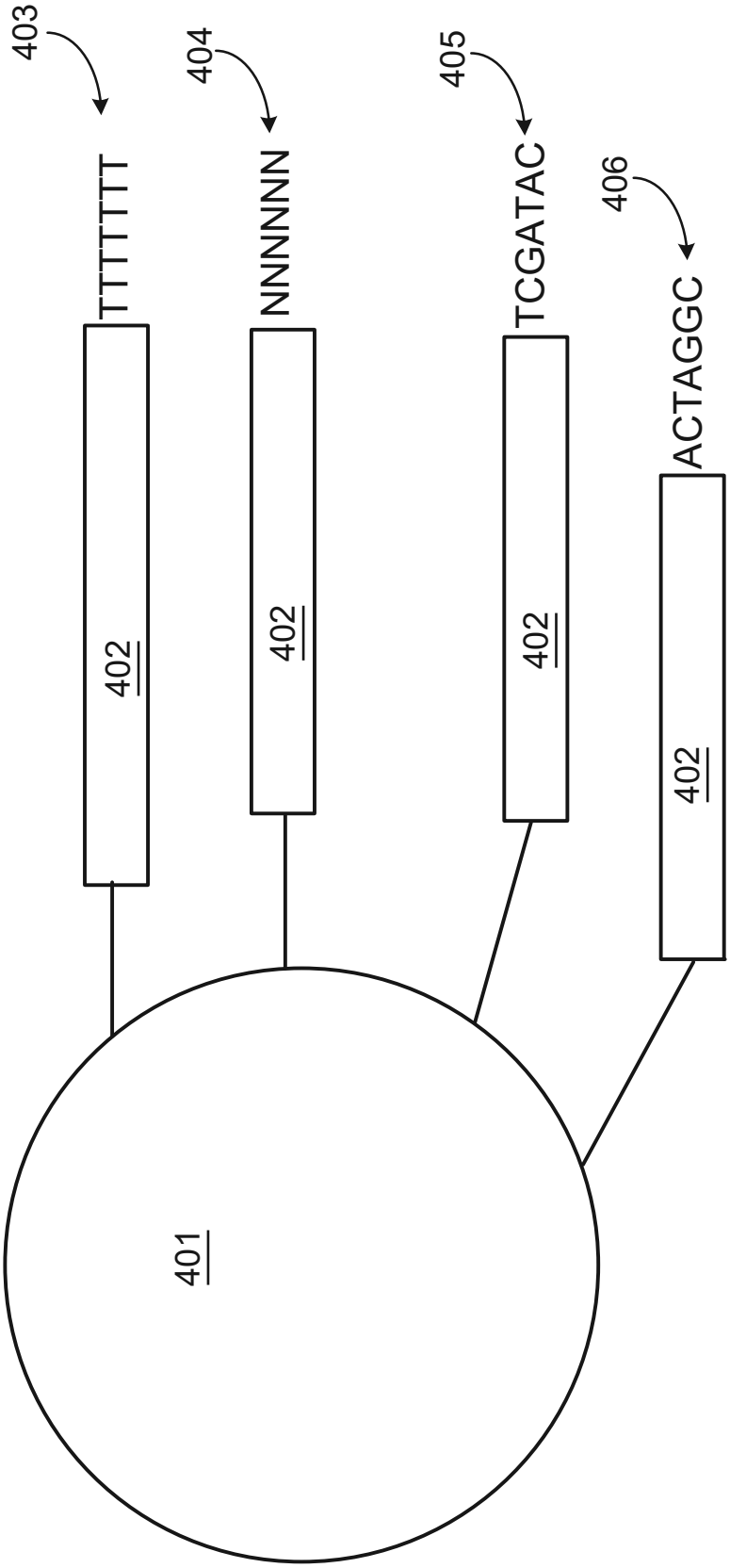

403
TTTTTTTT
404
NNNNNNN
405
TCGATAC
406
ACTAGGC
402
402
402
402
401

FIG. 4

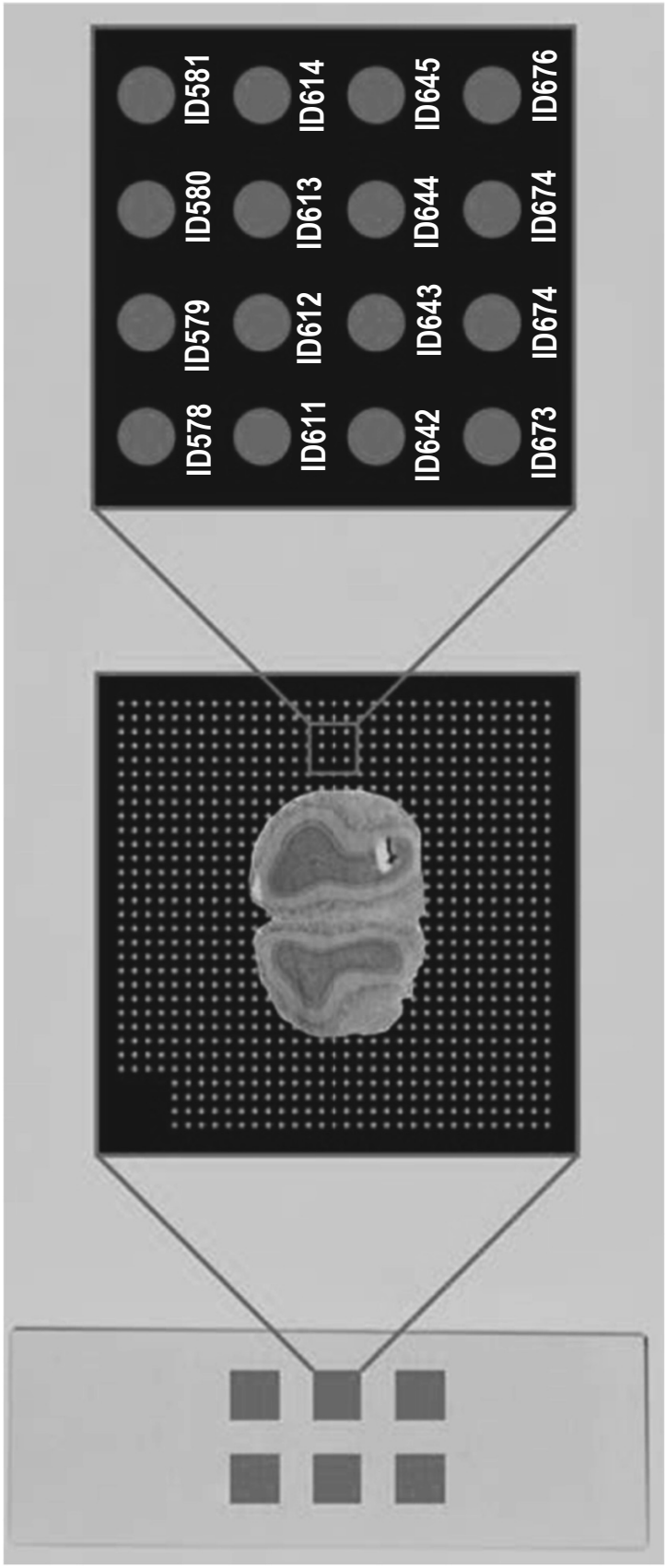

ID578
ID579
ID580
ID581
ID611
ID612
ID613
ID614
ID642
ID643
ID644
ID645
ID673
ID674
ID674
ID676

FIG. 5

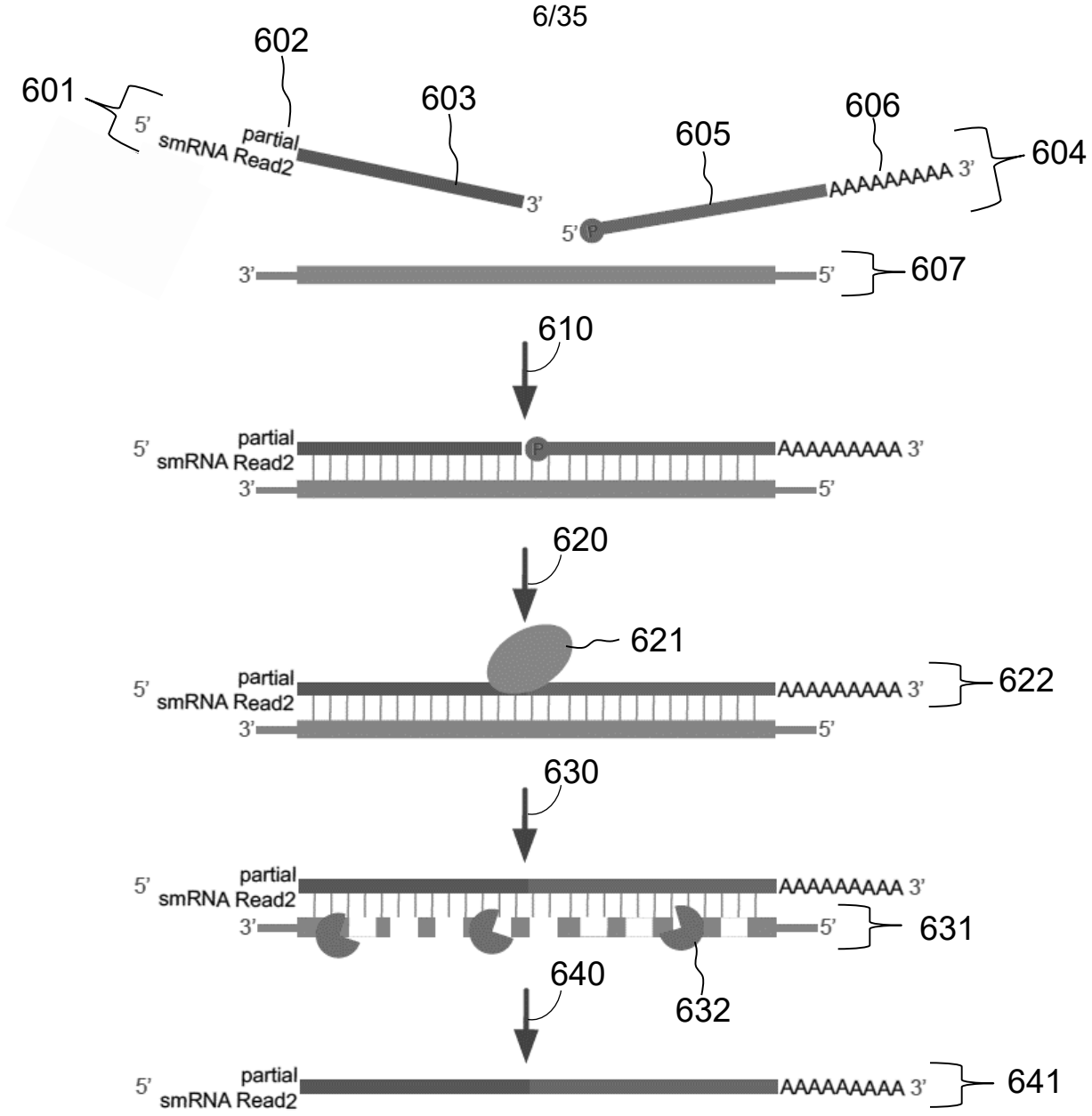

601
602
603
605
606
604
607
610
620
621
622
630
631
632
640
641
5'
partial
smRNA Read2
3'
5' P
AAAAAAAAA 3'
3'
5'
P

FIG. 6

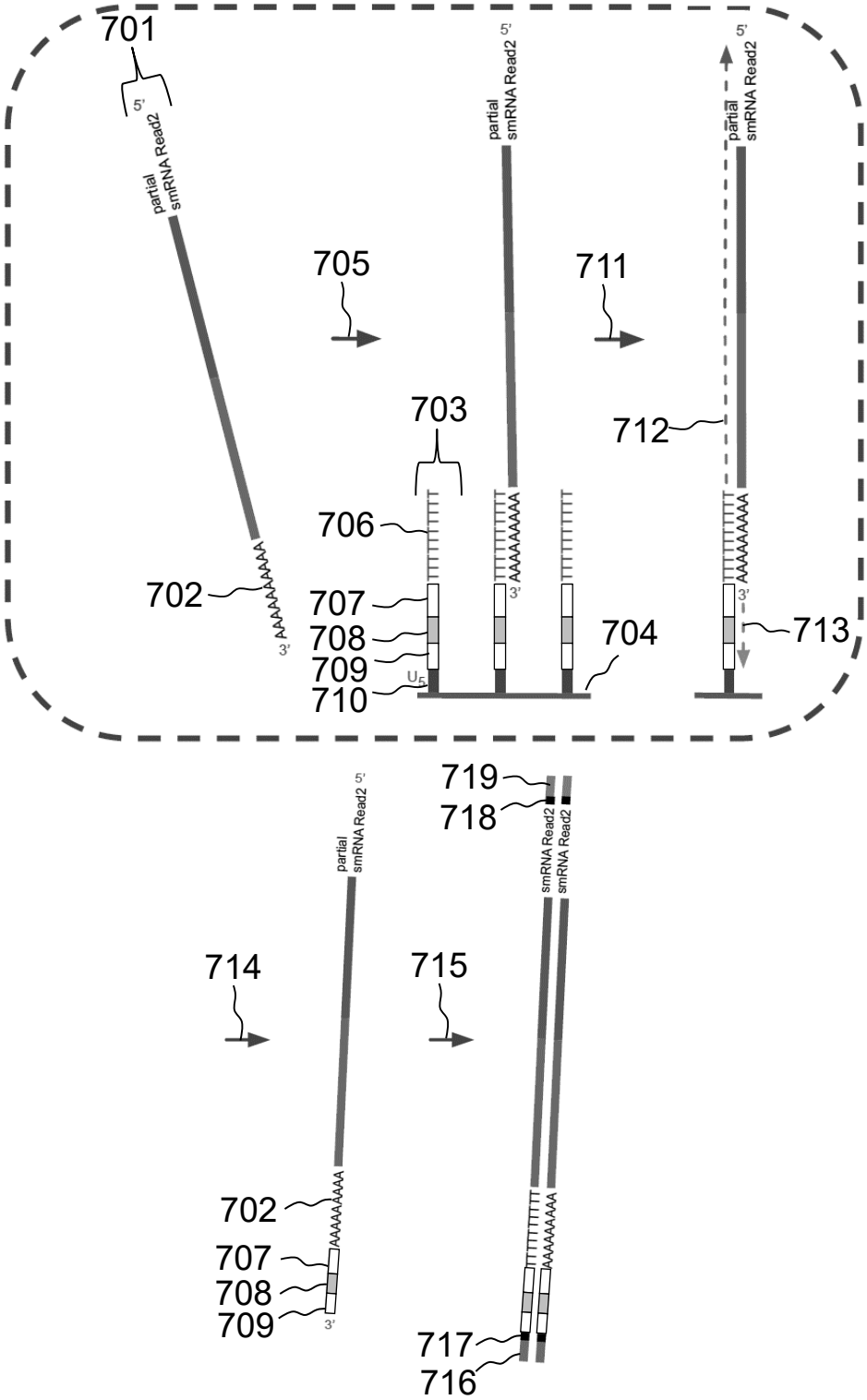

701
5'
partial smRNA Read2
702
AAAAAAAAAA
3'
705
703
706
707
708
709
710
U5
5'
partial smRNA Read2
AAAAAAAAAA
3'
704
711
712
5'
partial smRNA Read2
AAAAAAAAAA
3'
713
714
5'
partial smRNA Read2
702
AAAAAAAAAA
707
708
709
3'
715
719
718
smRNA Read2
smRNA Read2
AAAAAAAAAA
717
716

FIG. 7

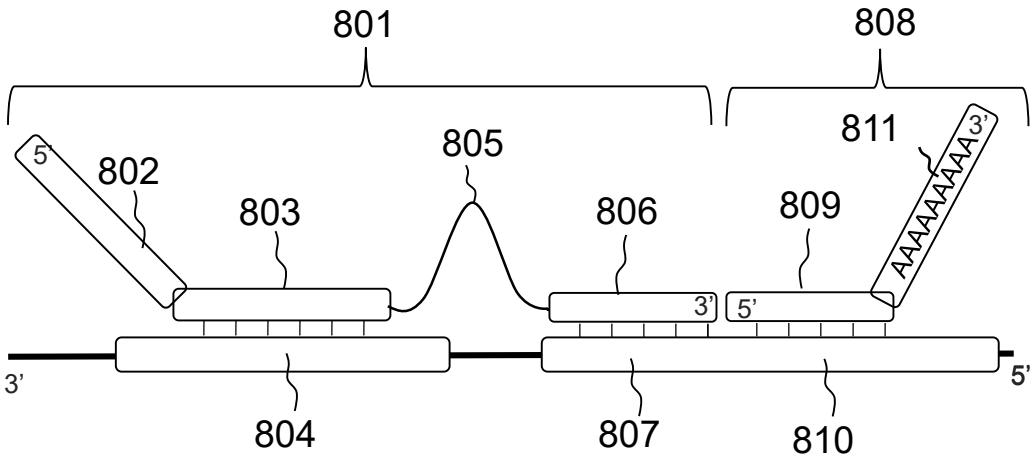

801
808
5'
802
803
805
806
809
811
3'
AAAAAAAAA
3'
5'
3'
5'
804
807
810

FIG. 8

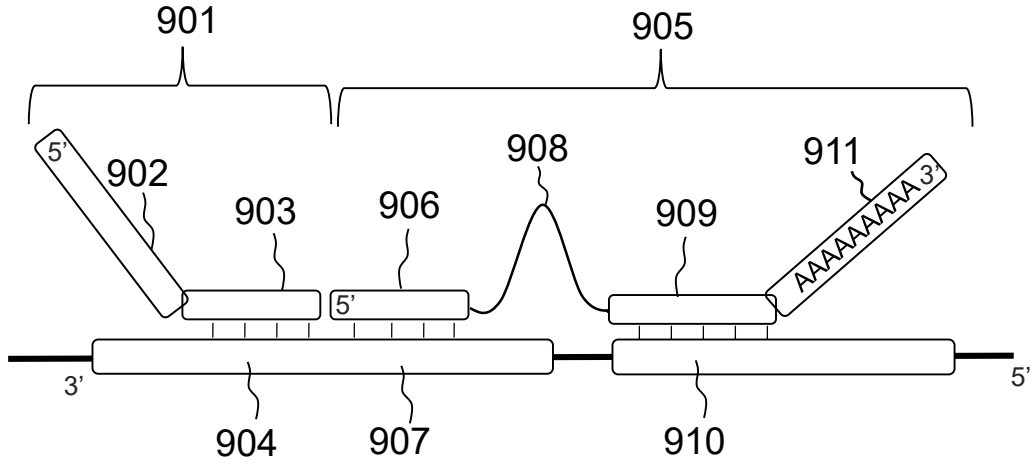

901
905
5'
902
903
906
908
909
911
3'
AAAAAAAAA
5'
3'
5'
904
907
910

FIG. 9

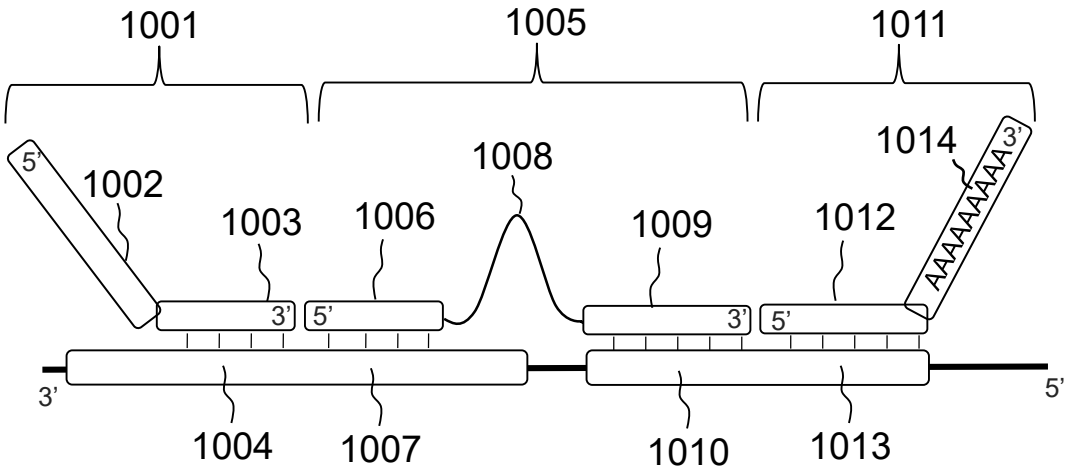

1001
1005
1011
5'
1002
1003
1006
1008
1009
1012
1014
3'
AAAAAAAA
3'
5'
3'
5'
3'
5'
1004
1007
1010
1013

FIG. 10

1111
1113
1110
1103
1105
1109
1112
1106
1101
1114
1108
1104
1102
1107

FIG. 11

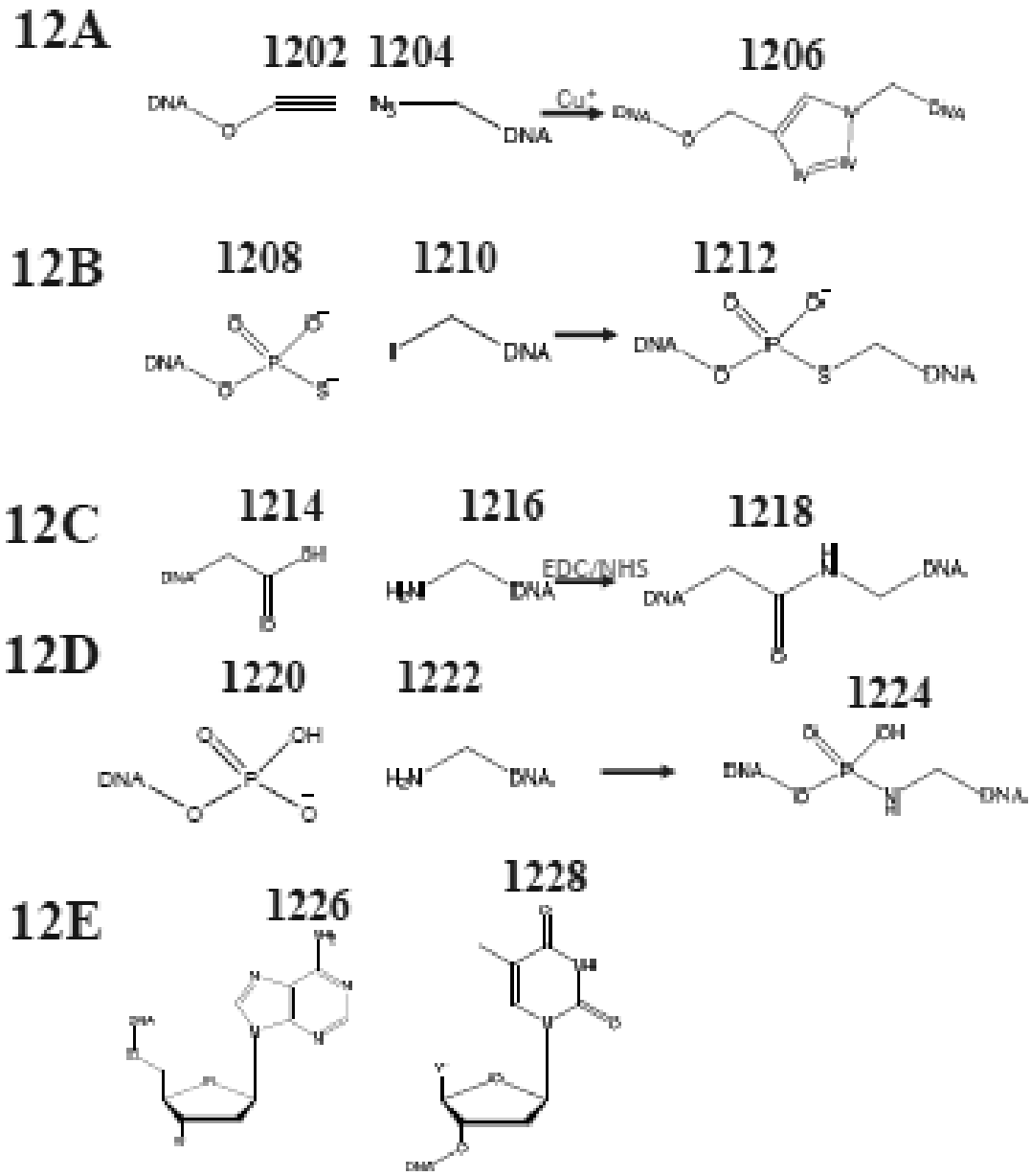

12A
1202
1204
1206
DNA
Cu+
12B
1208
1210
1212
DNA
12C
1214
1216
1218
EDC/NHS
DNA
12D
1220
1222
1224
DNA
12E
1226
1228

FIG. 12

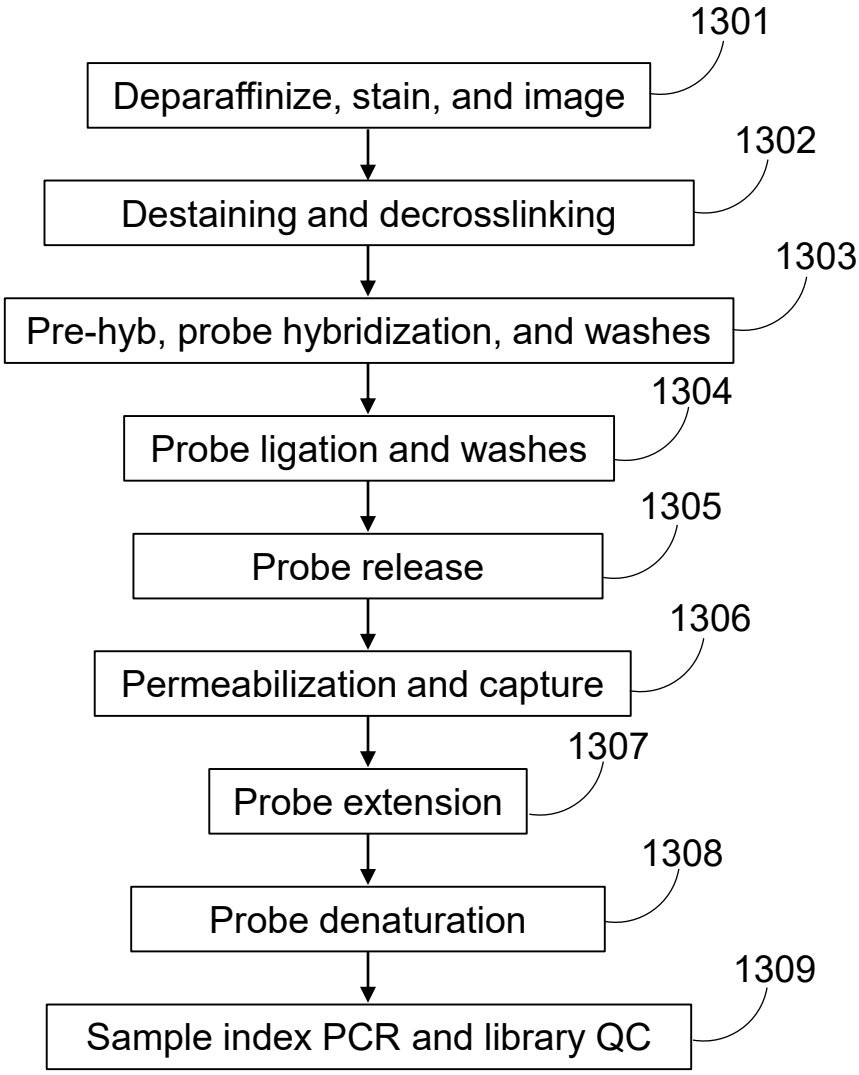

1301
Deparaffinize, stain, and image
1302
Destaining and decrosslinking
1303
Pre-hyb, probe hybridization, and washes
1304
Probe ligation and washes
1305
Probe release
1306
Permeabilization and capture
1307
Probe extension
1308
Probe denaturation
1309
Sample index PCR and library QC

FIG. 13

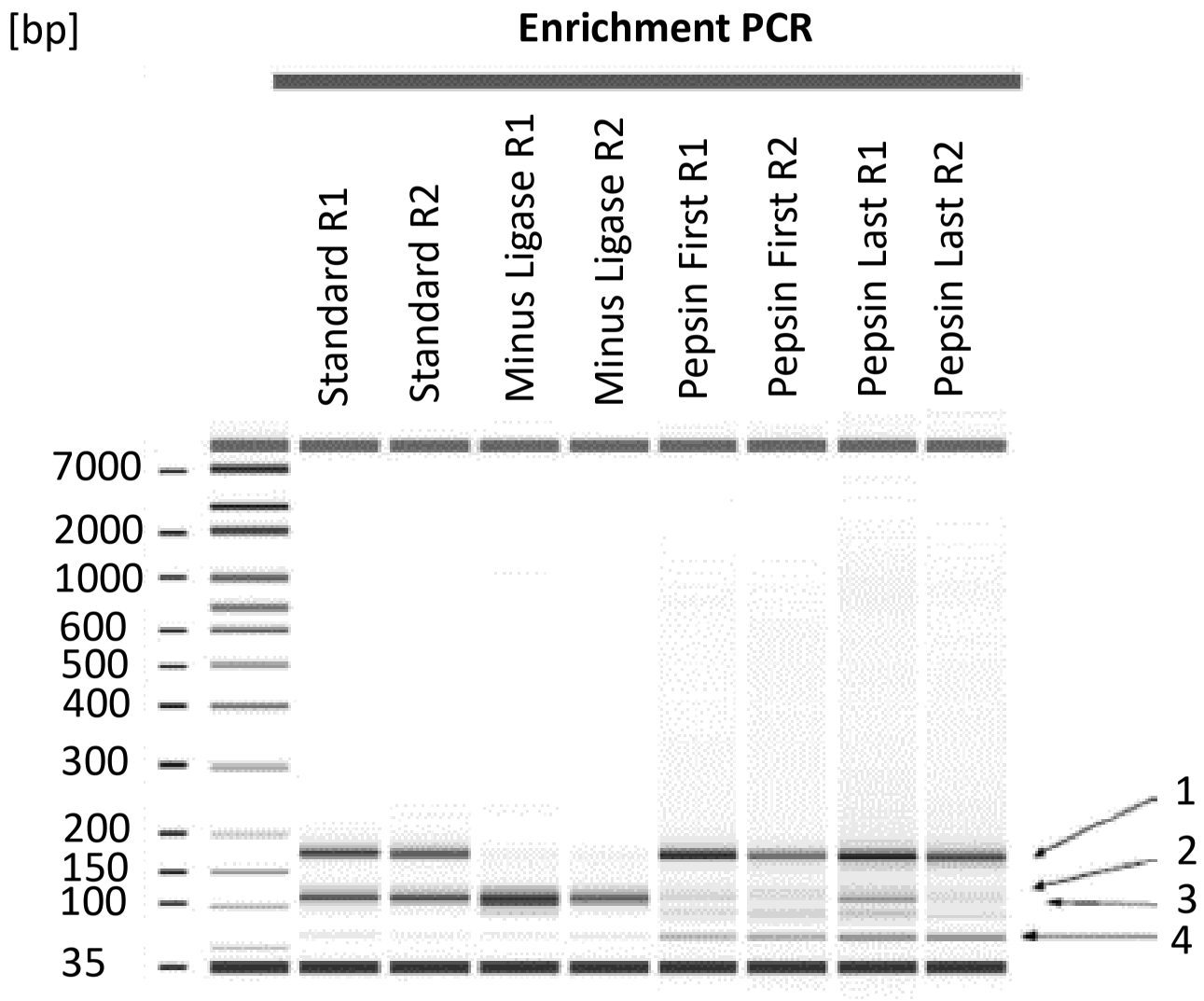

[bp]
Enrichment PCR
Standard R1
Standard R2
Minus Ligase R1
Minus Ligase R2
Pepsin First R1
Pepsin First R2
Pepsin Last R1
Pepsin Last R2
7000
2000
1000
600
500
400
300
200
150
100
35
1
2
3
4

FIG. 14

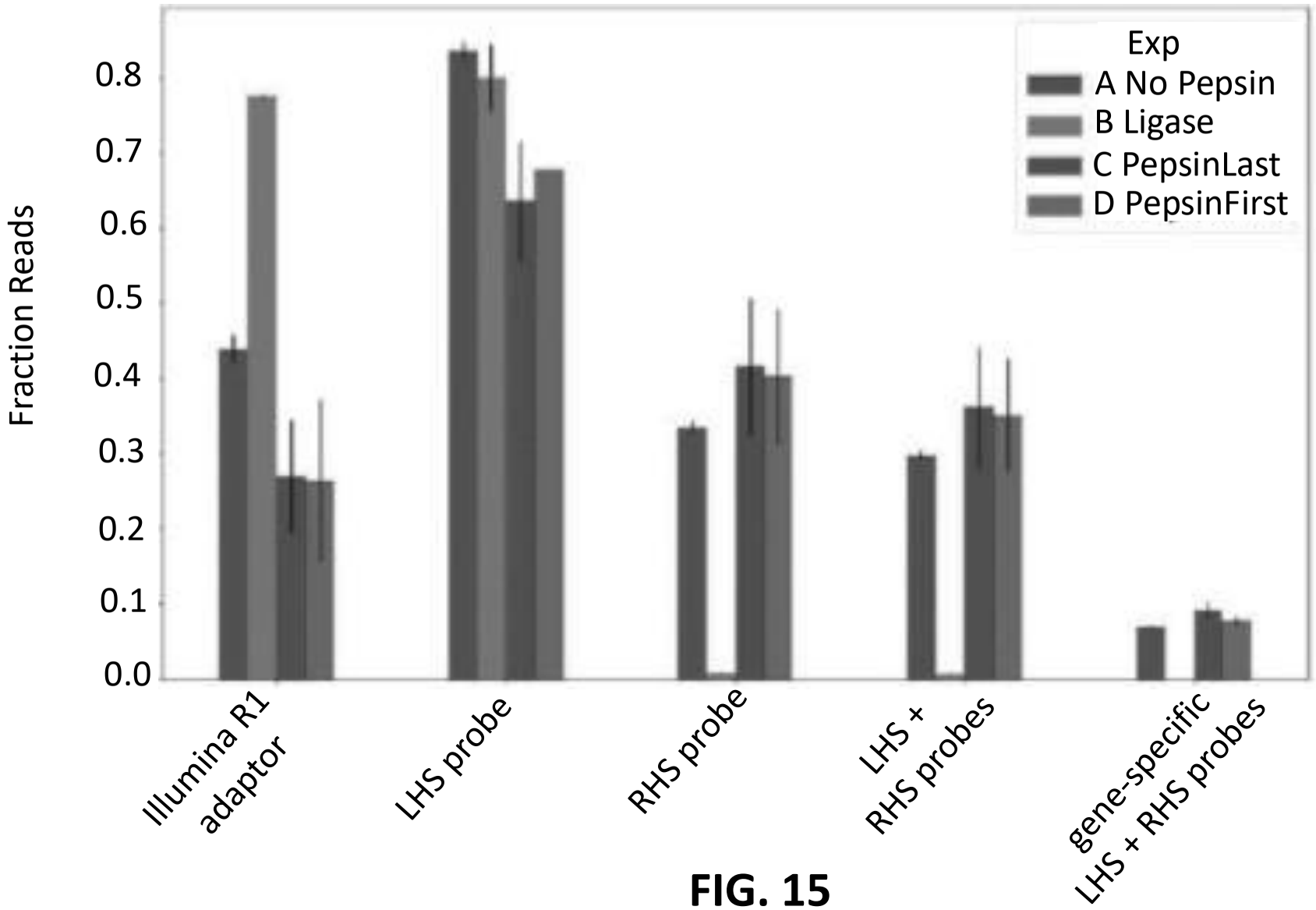

Exp
A No Pepsin
B Ligase
C PepsinLast
D PepsinFirst
Fraction Reads
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Illumina R1 adaptor
LHS probe
RHS probe
LHS + RHS probes
gene-specific LHS + RHS probes

FIG. 15

A Standard

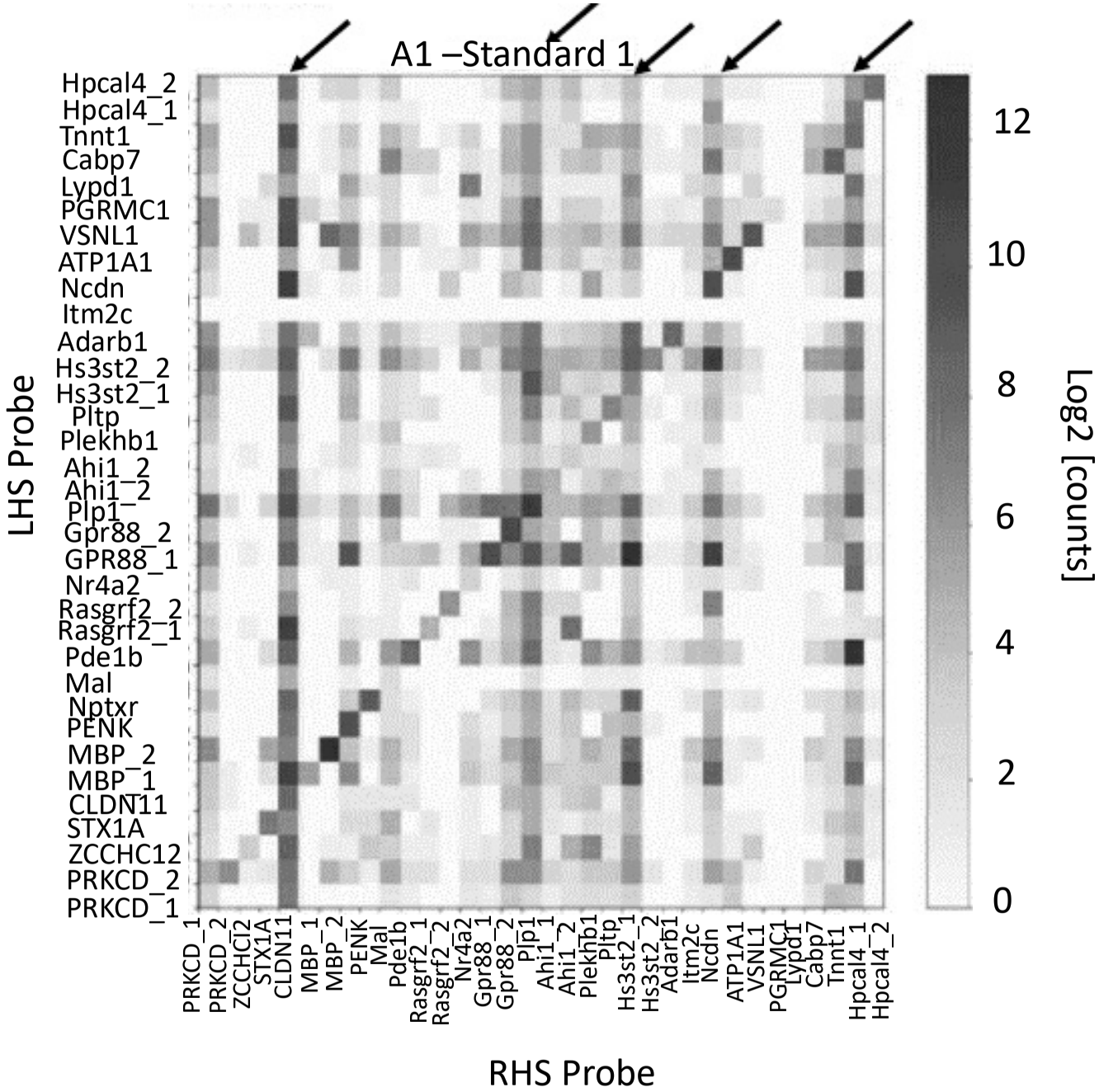


FIG. 16A

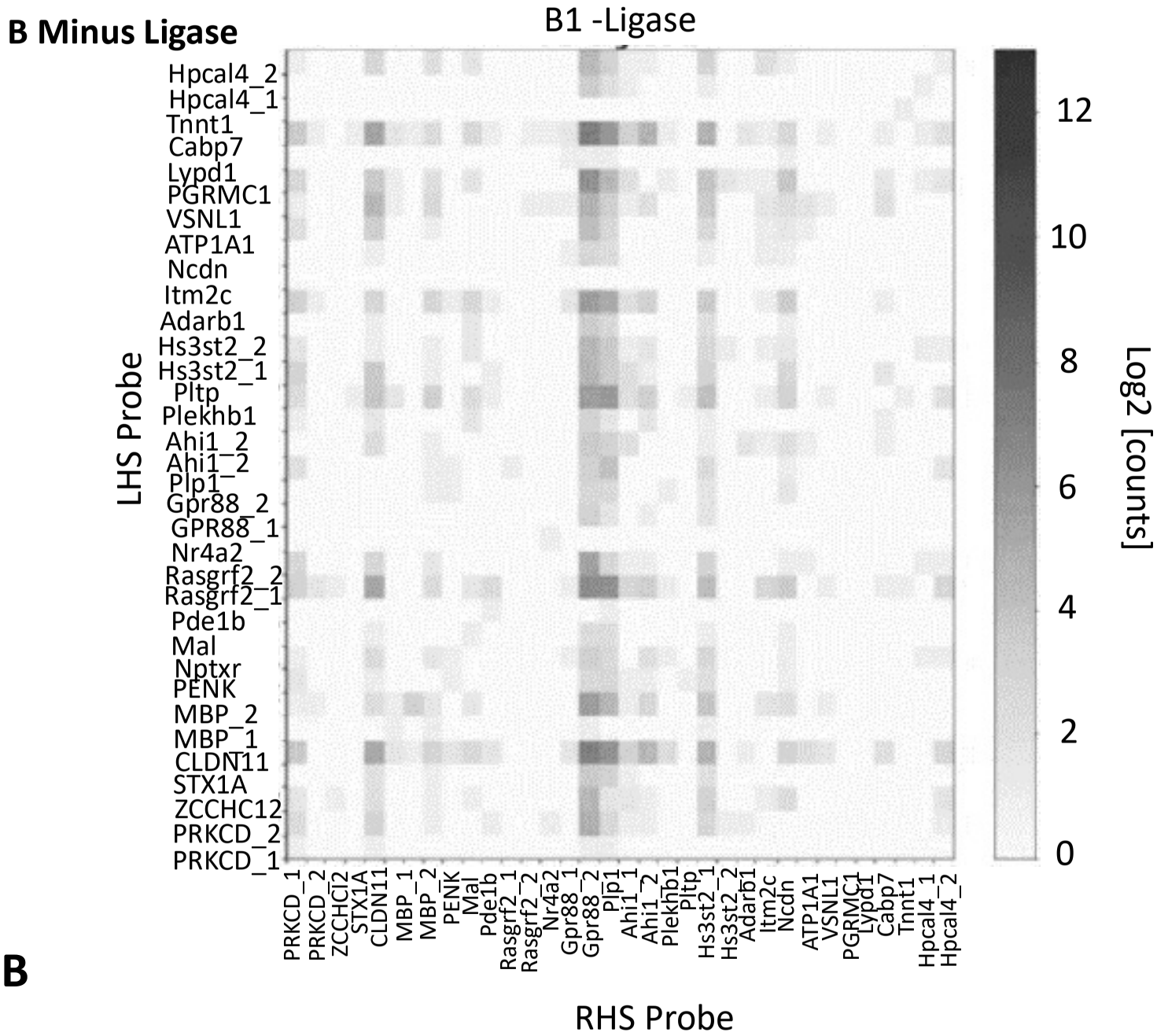

B Minus Ligase
B1 -Ligase
LHS Probe
Hpcal4_2
Hpcal4_1
Tnnt1
Cabp7
Lypd1
PGRMC1
VSNL1
ATP1A1
Ncdn
Itm2c
Adarb1
Hs3st2_2
Hs3st2_1
Pltp
Plekhb1
Ahi1_2
Ahi1_2
Plp1
Gpr88_2
GPR88_1
Nr4a2
Rasgrf2_2
Rasgrf2_1
Pde1b
Mal
Nptxr
PENK
MBP_2
MBP_1
CLDN11
STX1A
ZCCHC12
PRKCD_2
PRKCD_1
PRKCD_1
PRKCD_2
ZCCHC12
STX1A
CLDN11
MBP_1
MBP_2
PENK
Mal
Pde1b
Rasgrf2_1
Rasgrf2_2
Nr4a2
Gpr88_1
Gpr88_2
Plp1
Ahi1_1
Ahi1_2
Plekhb1
Pltp
Hs3st2_1
Hs3st2_2
Adarb1
Itm2c
Ncdn
ATP1A1
VSNL1
PGRMC1
Lypd1
Cabp7
Tnnt1
Hpcal4_1
Hpcal4_2
RHS Probe
12
10
8
6
4
2
0
Log2 [counts]

FIG. 16B

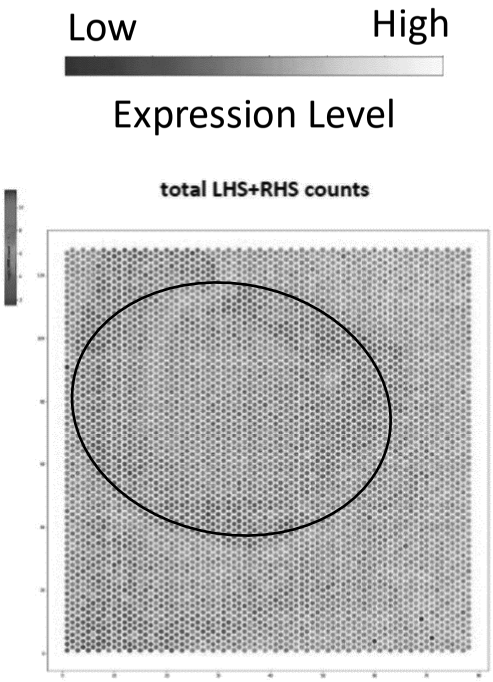


FIG. 17A

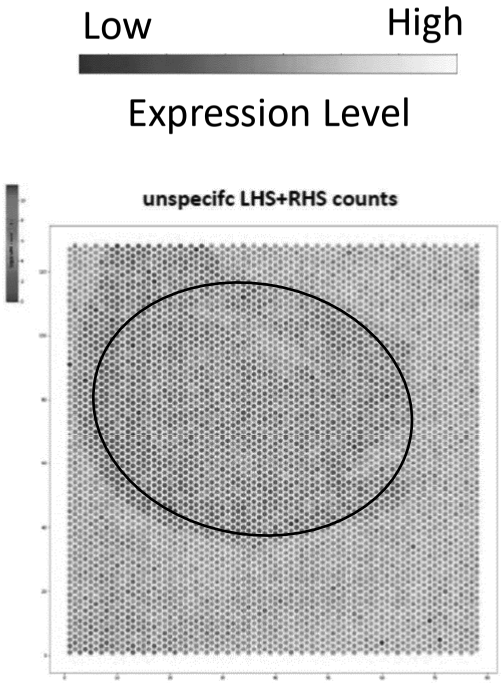


FIG. 17B

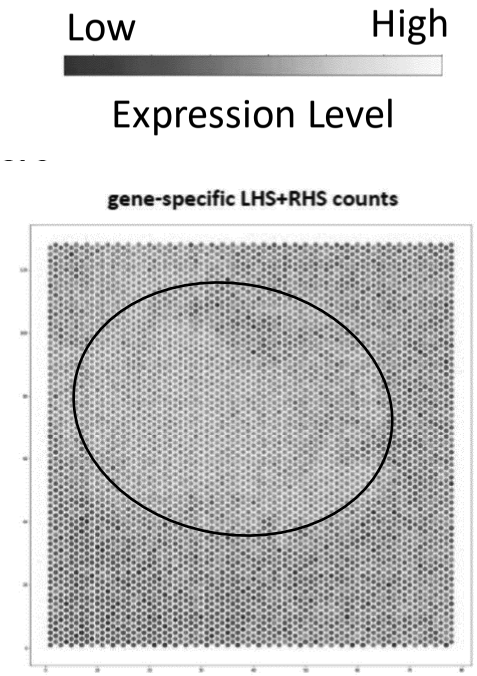


FIG. 17C

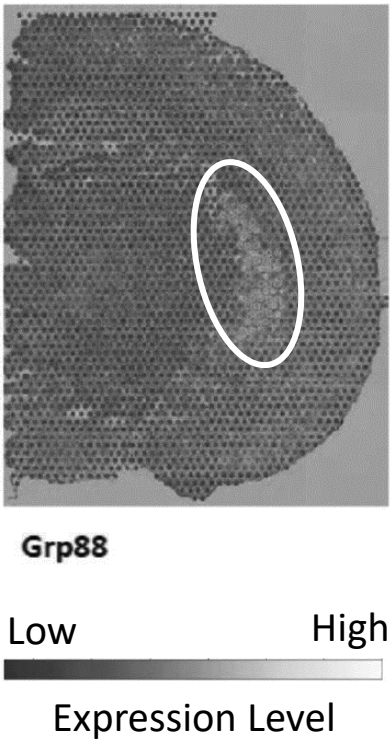


FIG. 18A

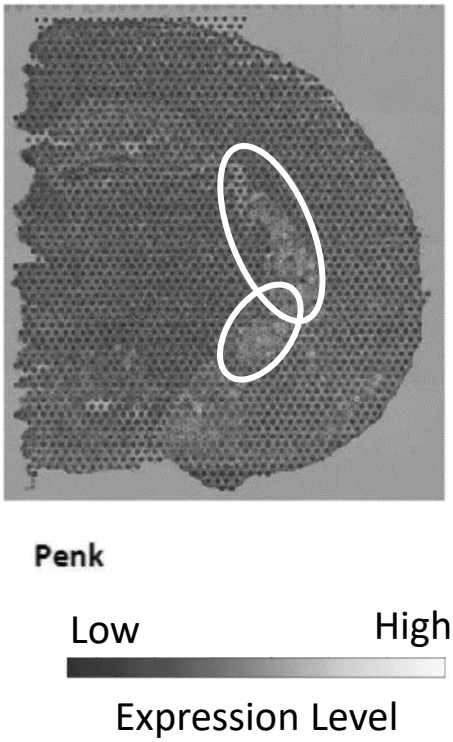
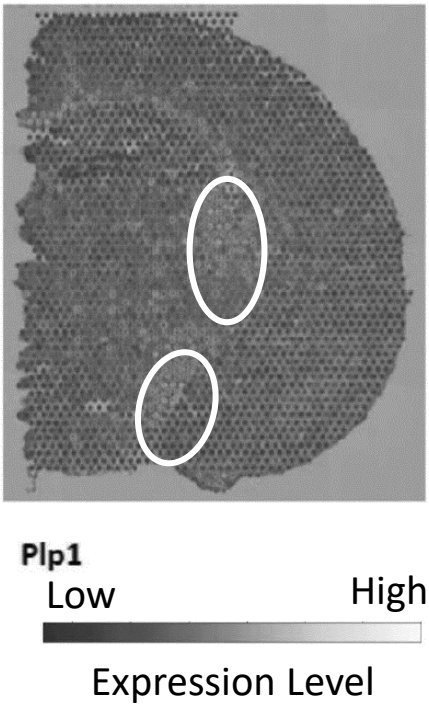
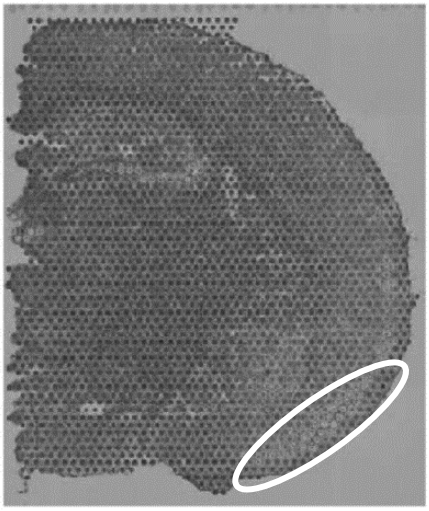


FIG. 18B

FIG. 18C



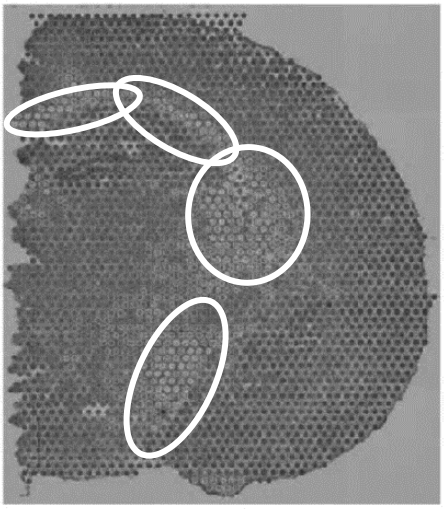


Nptrx

Low High

Expression Level

FIG. 18D



Mbp2

Low High

Expression Level

FIG. 18E

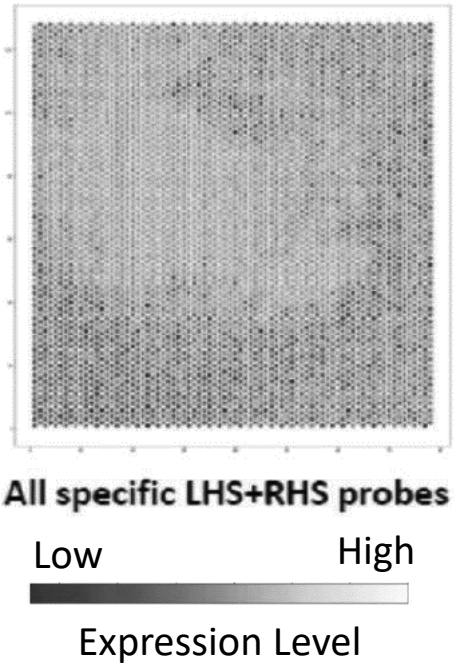


FIG. 19A

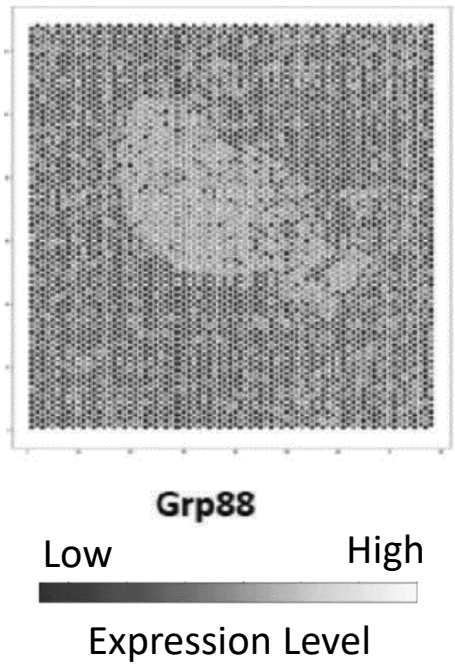


FIG. 19B

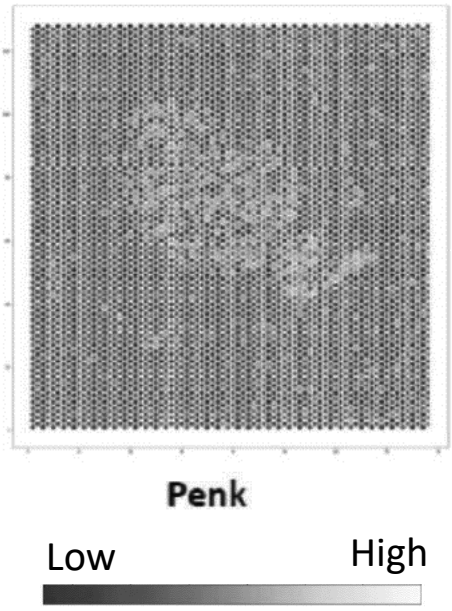


FIG. 19C

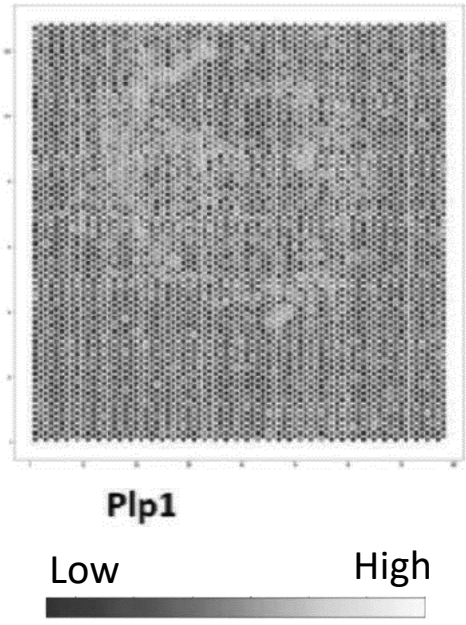


FIG. 19D

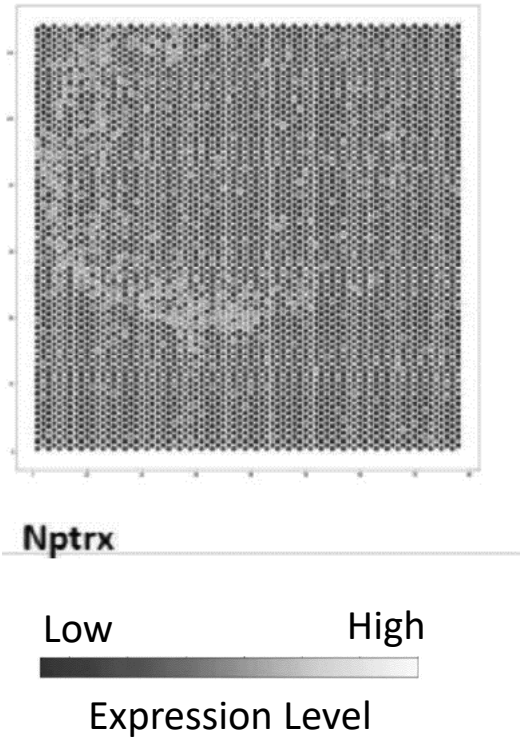


FIG. 19E

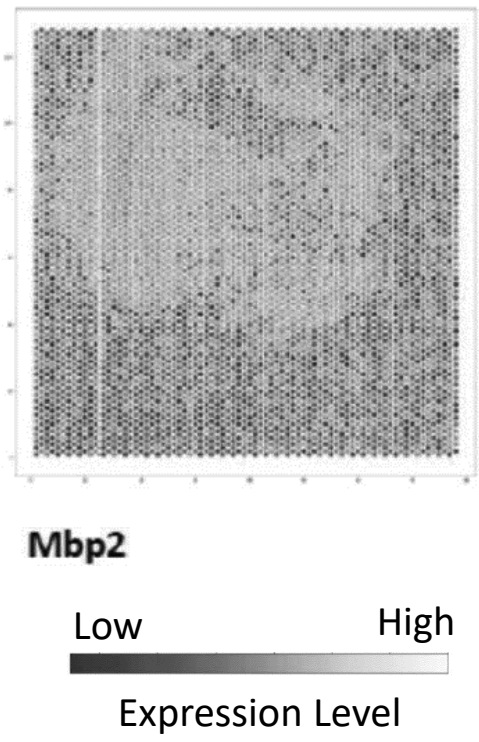


FIG. 19F

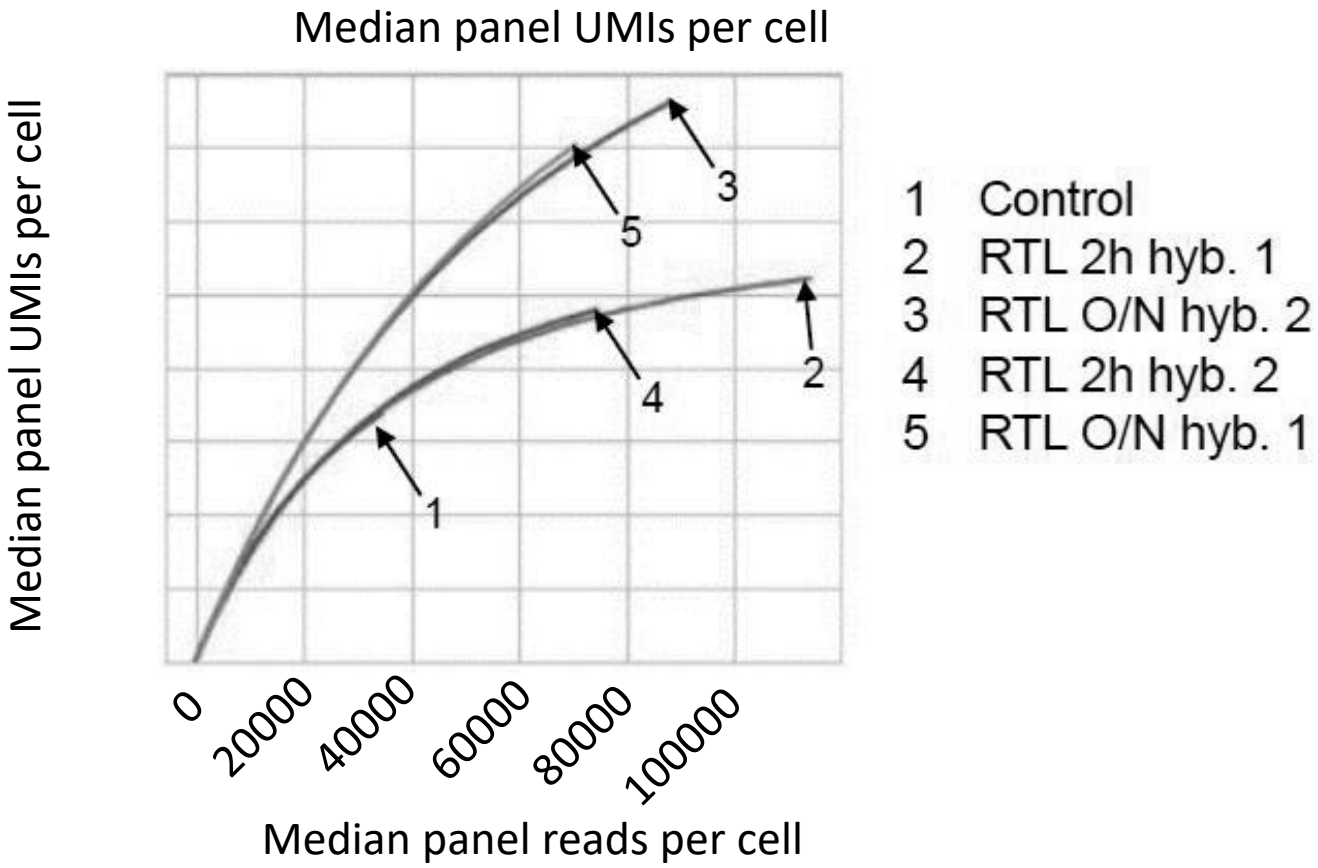


FIG. 20A

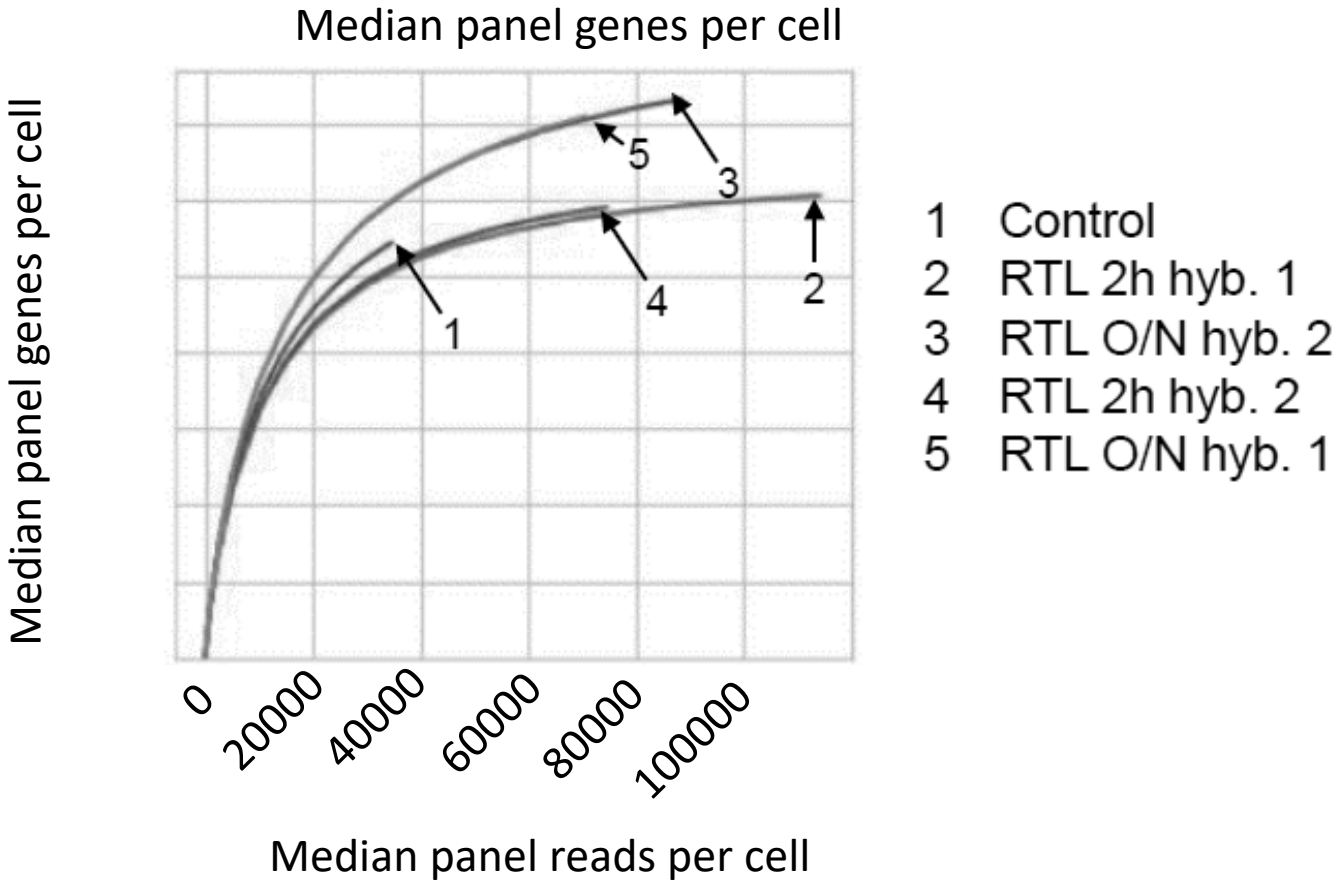


FIG. 20B

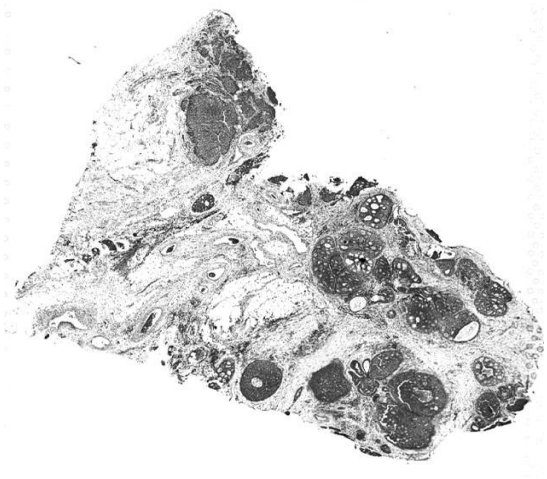


FIG. 21A

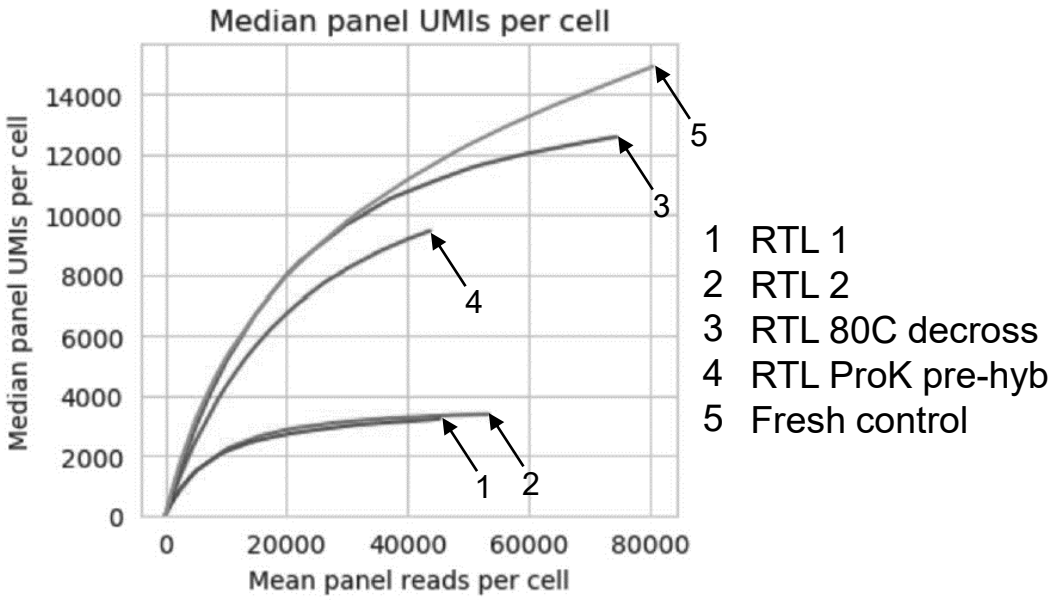


FIG. 21B

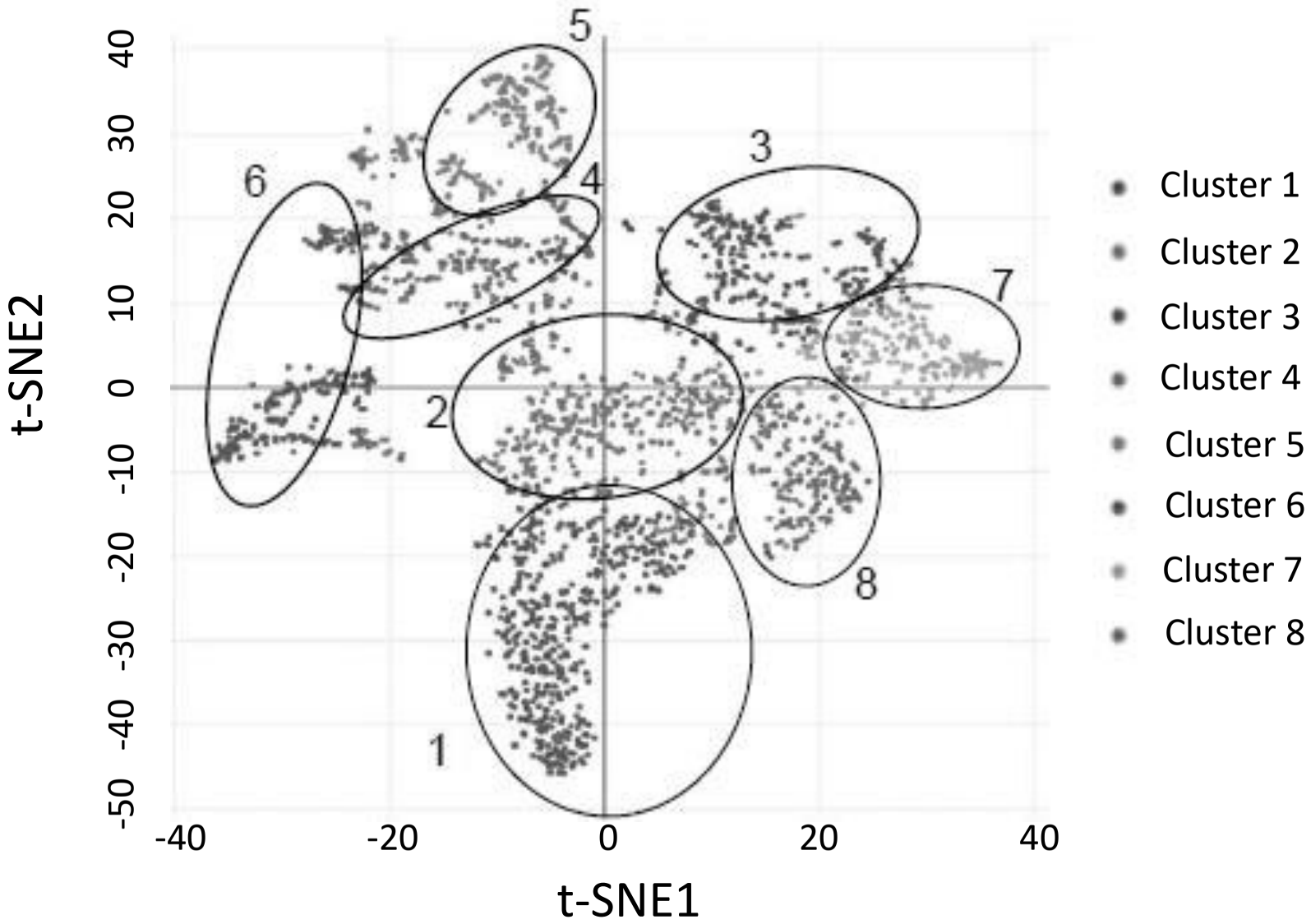

t-SNE2
40
30
20
10
0
-10
-20
-30
-40
-50
-40
-20
0
20
40
t-SNE1
1
2
3
4
5
6
7
8
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8

FIG. 21C

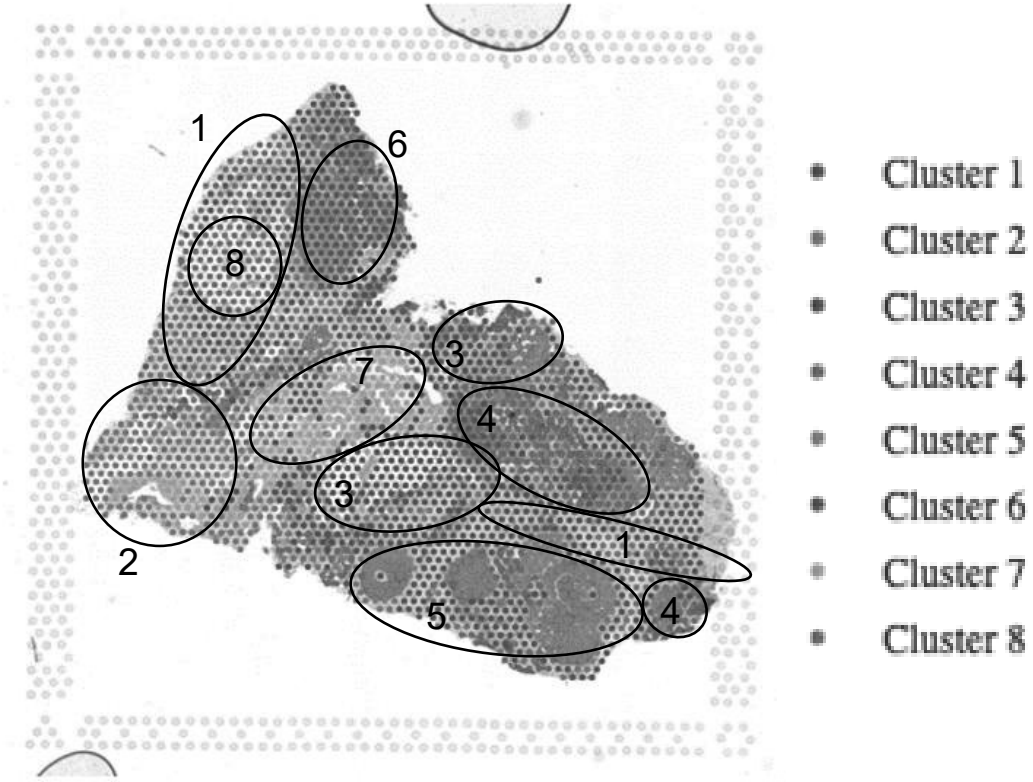

1
6
8
7
3
4
3
2
1
5
4
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8

FIG. 21D

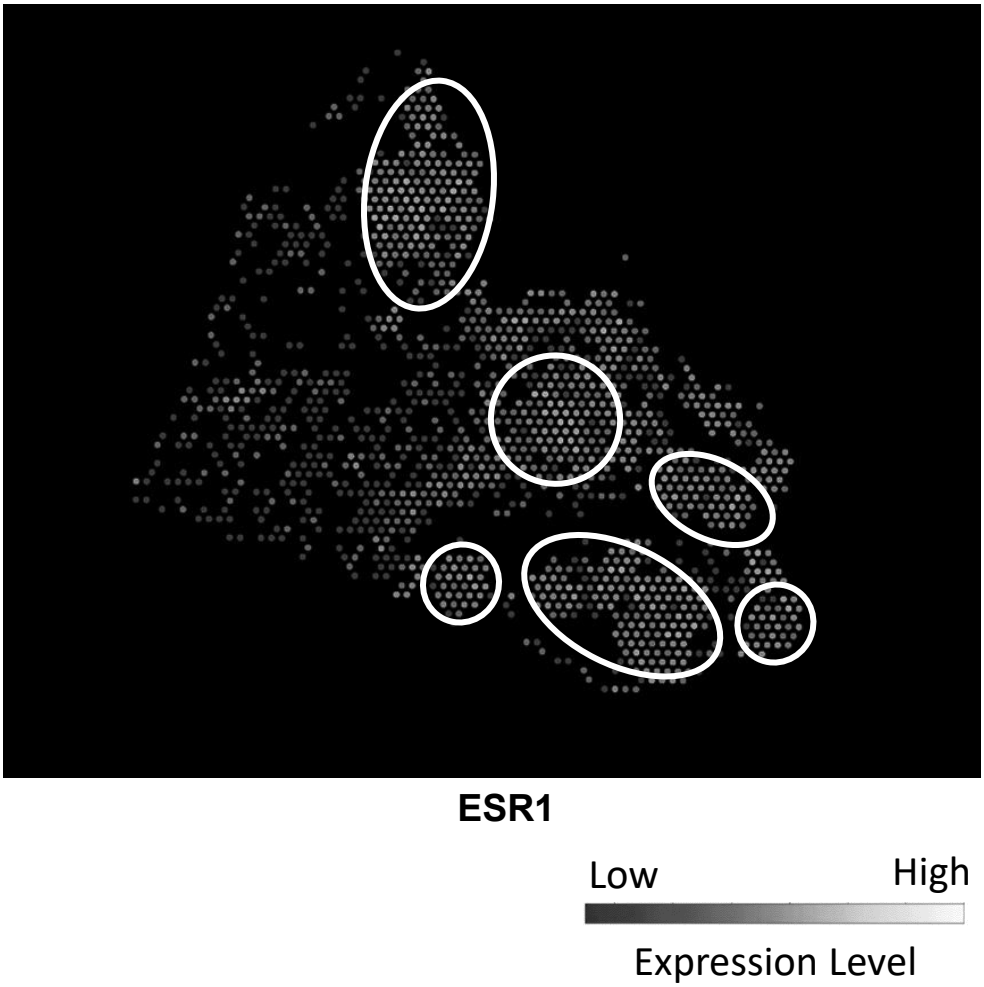
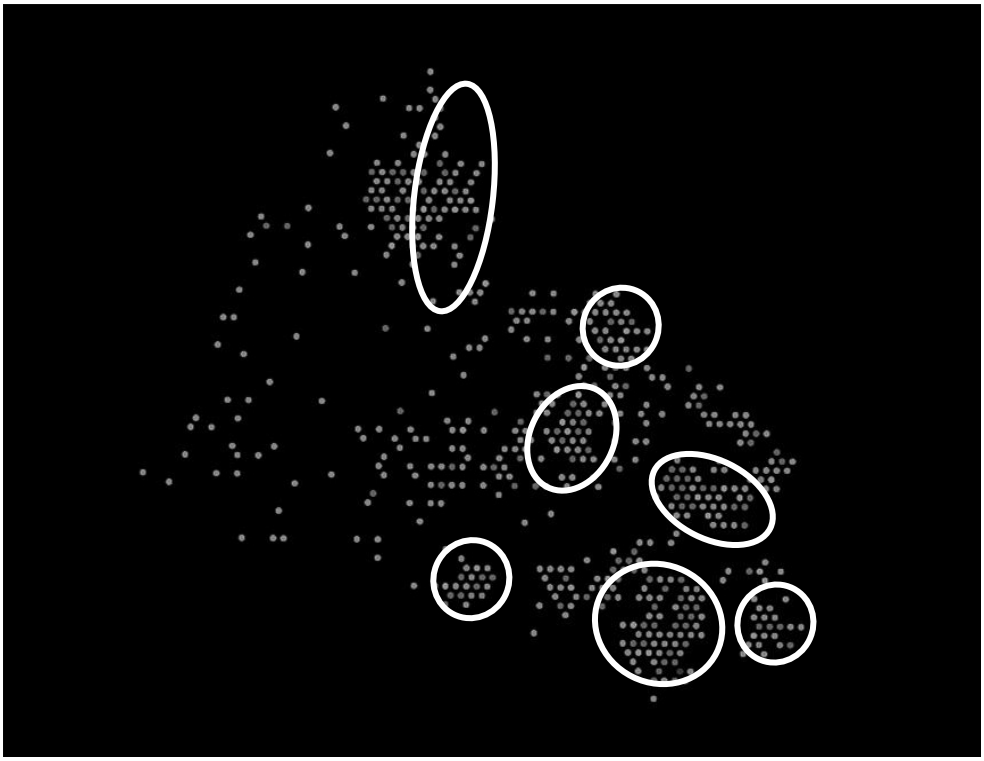
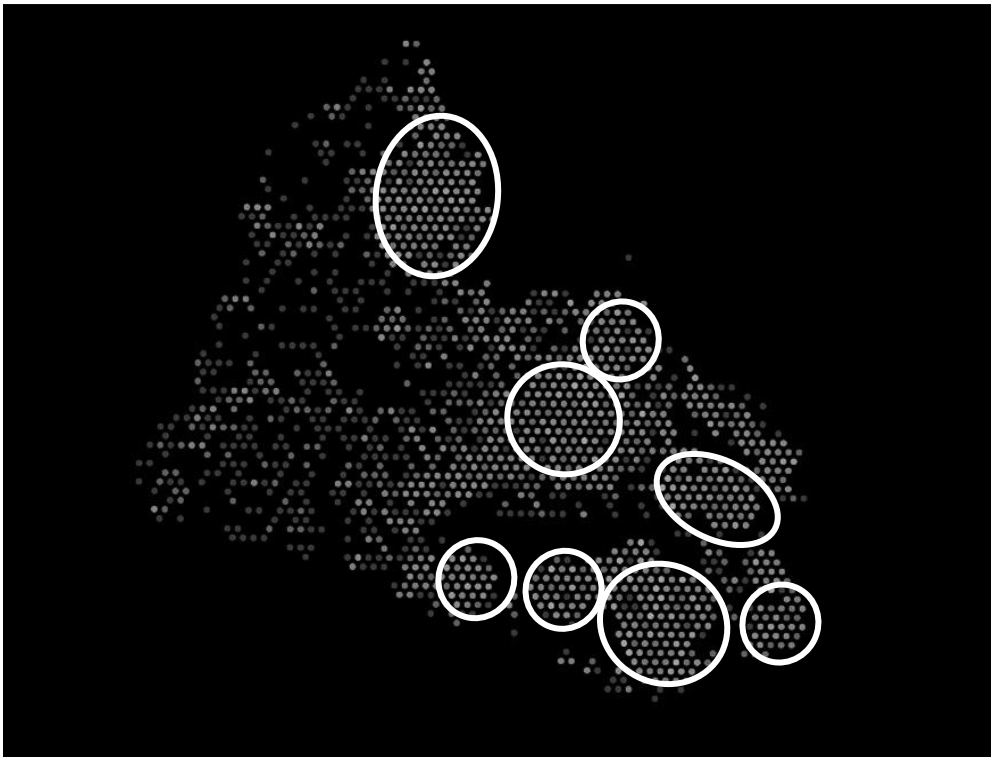


FIG. 21E



PGR

FIG. 21F



ERBB2 / HER2

FIG. 21G

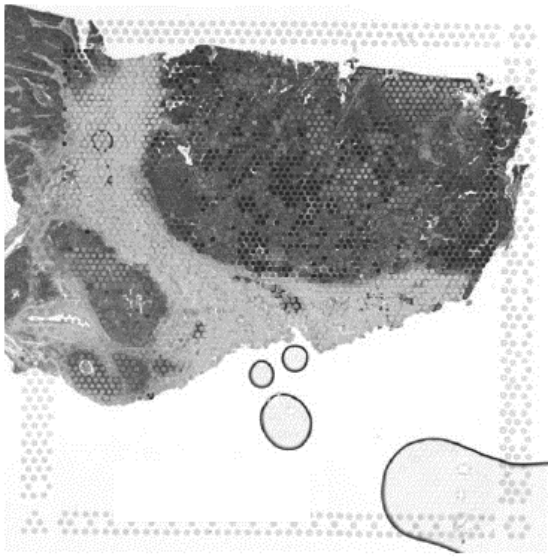


FIG. 22A

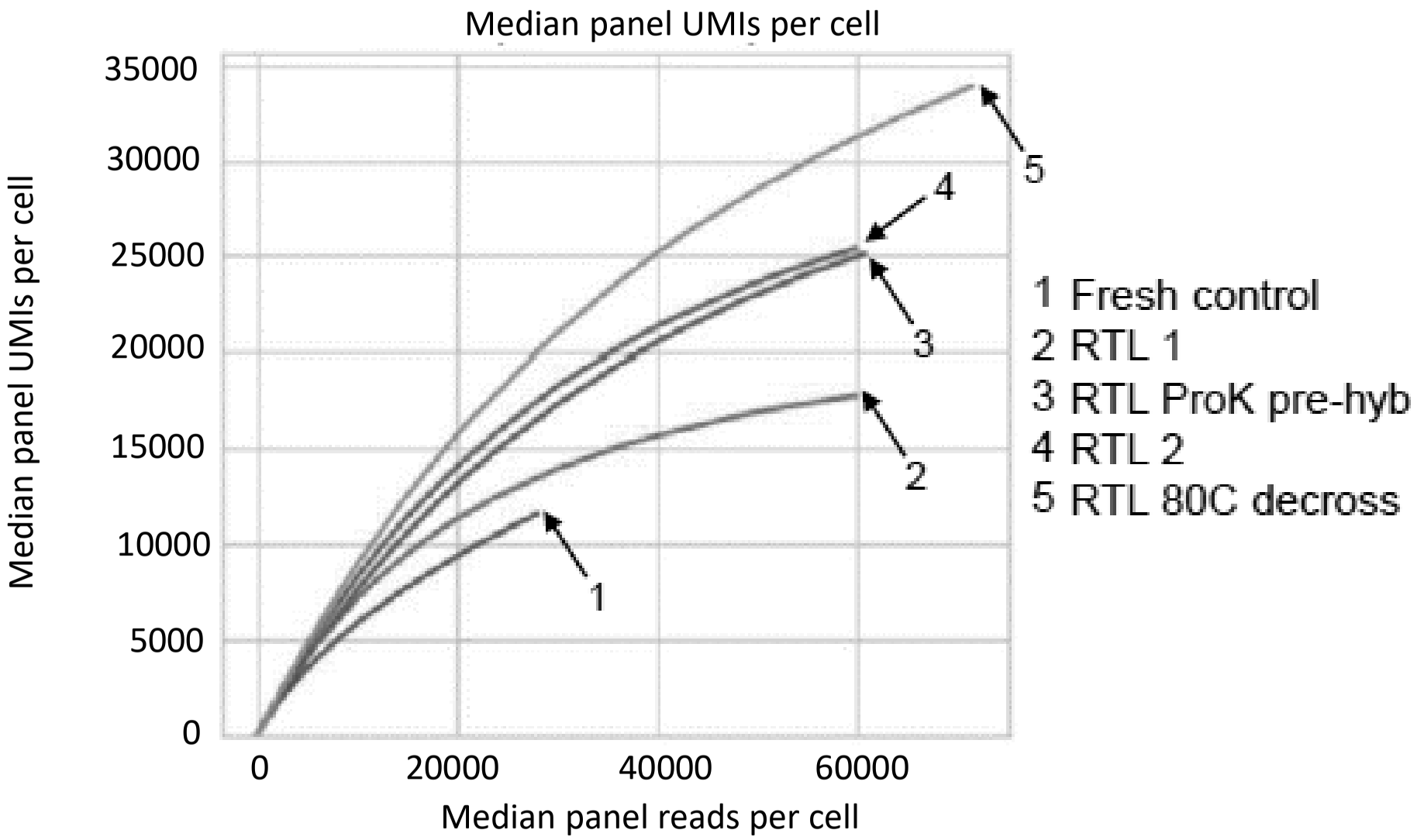

Median panel UMIs per cell
35000
30000
25000
20000
15000
10000
5000
0
0
20000
40000
60000
Median panel reads per cell
Median panel UMIs per cell
1
2
3
4
5
1 Fresh control
2 RTL 1
3 RTL ProK pre-hyb
4 RTL 2
5 RTL 80C decross

FIG. 22B

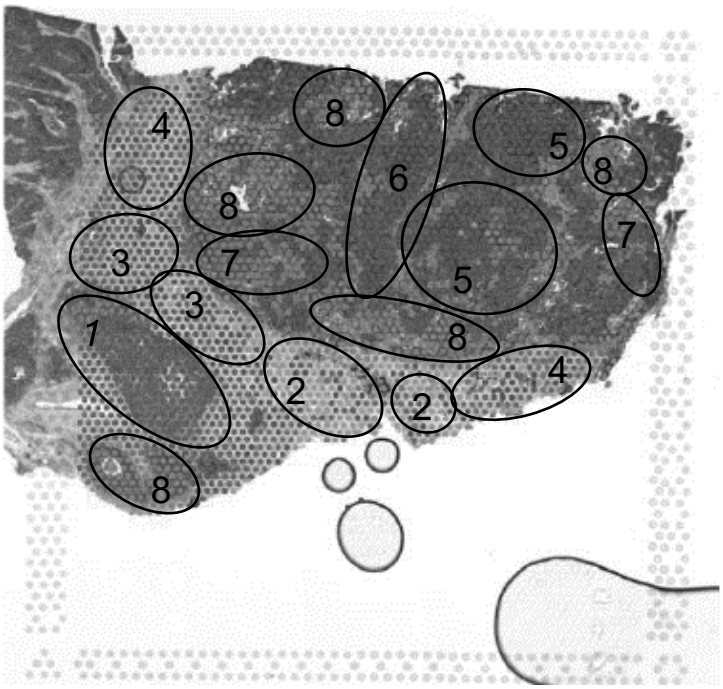


FIG. 22C

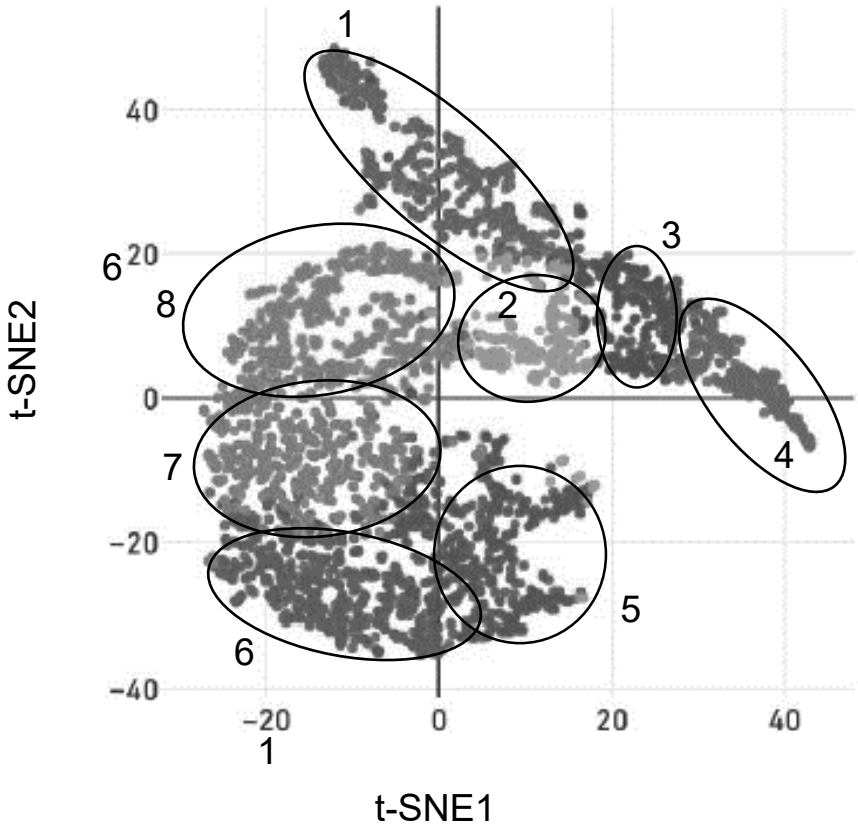


FIG. 22D

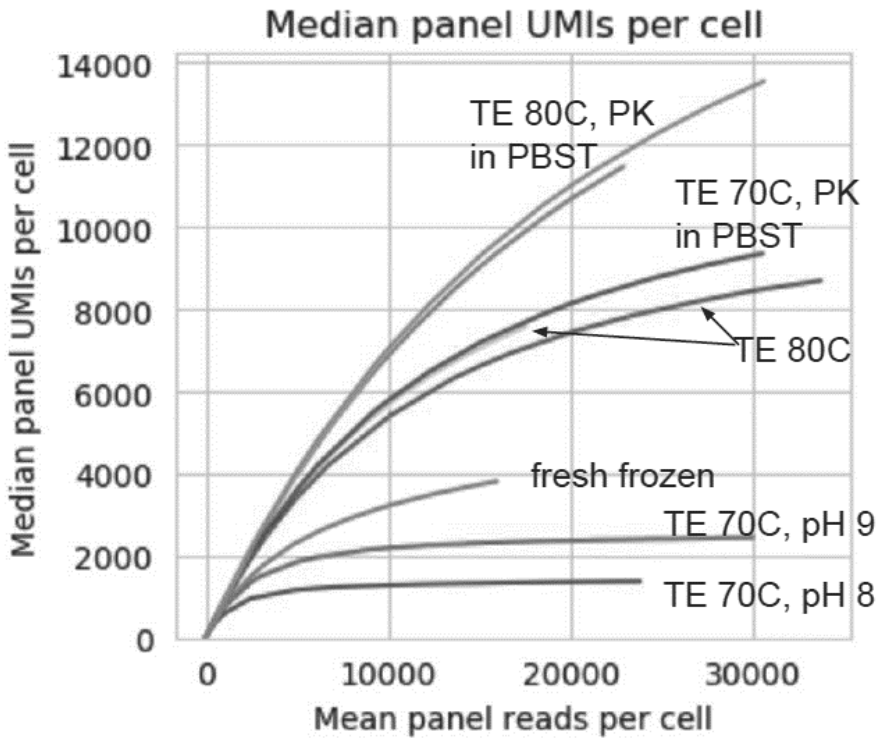
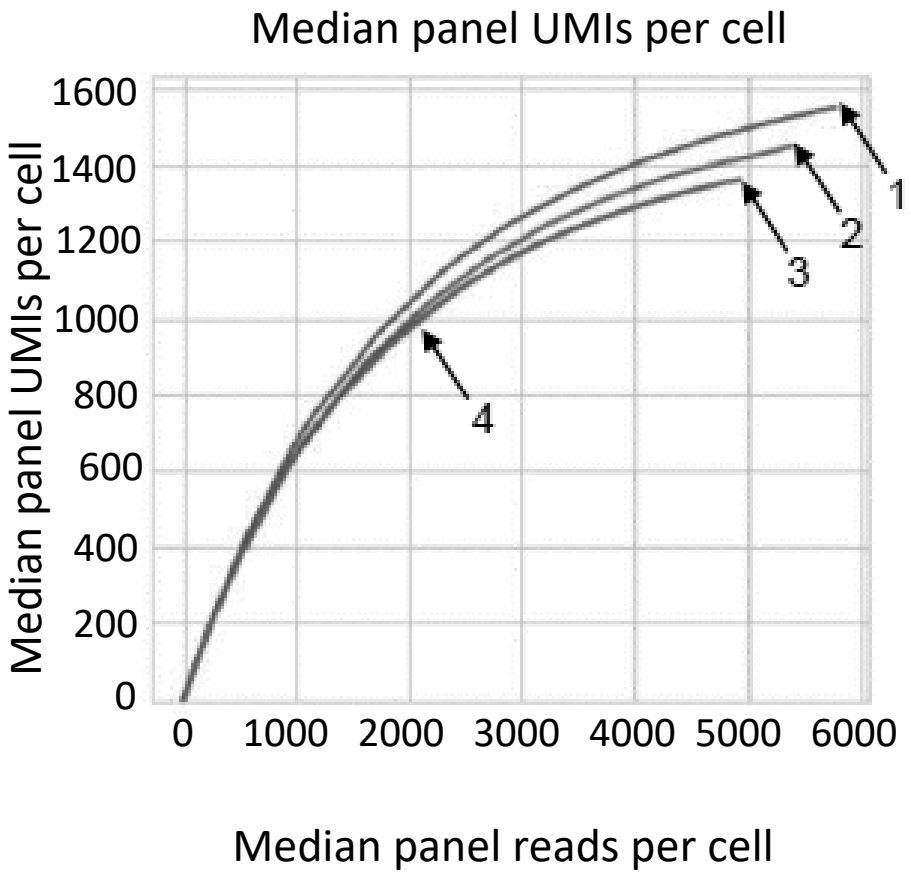

Median panel UMIs per cell
Median panel UMIs per cell
14000
12000
10000
8000
6000
4000
2000
0
0
10000
20000
30000
Mean panel reads per cell
TE 80C, PK
in PBST
TE 70C, PK
in PBST
TE 80C
fresh frozen
TE 70C, pH 9
TE 70C, pH 8

FIG. 23


Median panel UMIs per cell
Median panel UMIs per cell
1600
1400
1200
1000
800
600
400
200
0
0
1000
2000
3000
4000
5000
6000
1
2
3
4
Median panel reads per cell

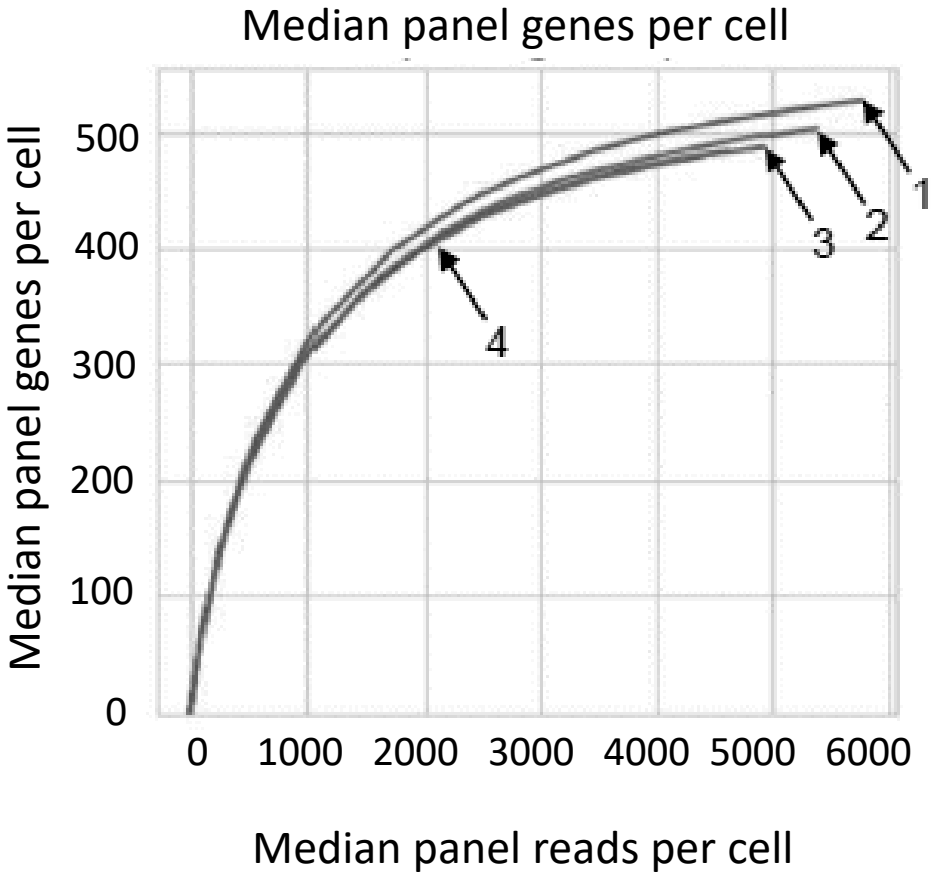
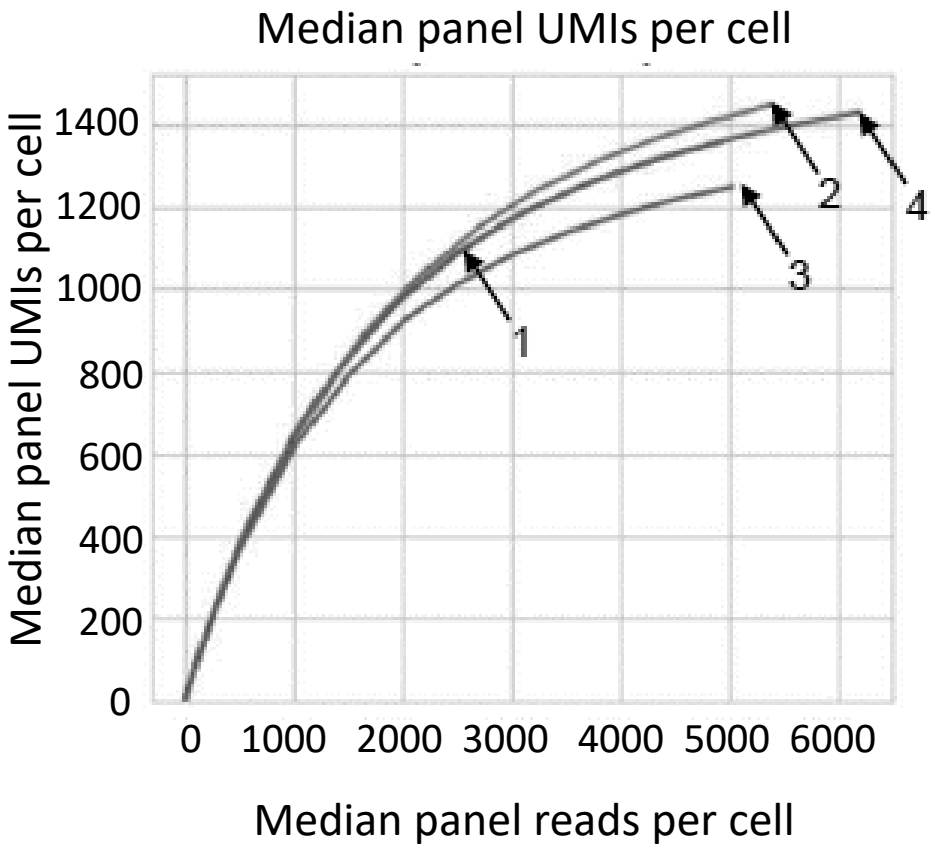

Median panel genes per cell
Median panel genes per cell
500
400
300
200
100
0
0
1000
2000
3000
4000
5000
6000
1
2
3
4
Median panel reads per cell

FIG. 24


Median panel UMIs per cell
Median panel UMIs per cell
1400
1200
1000
800
600
400
200
0
0
1000
2000
3000
4000
5000
6000
Median panel reads per cell
1
2
3
4

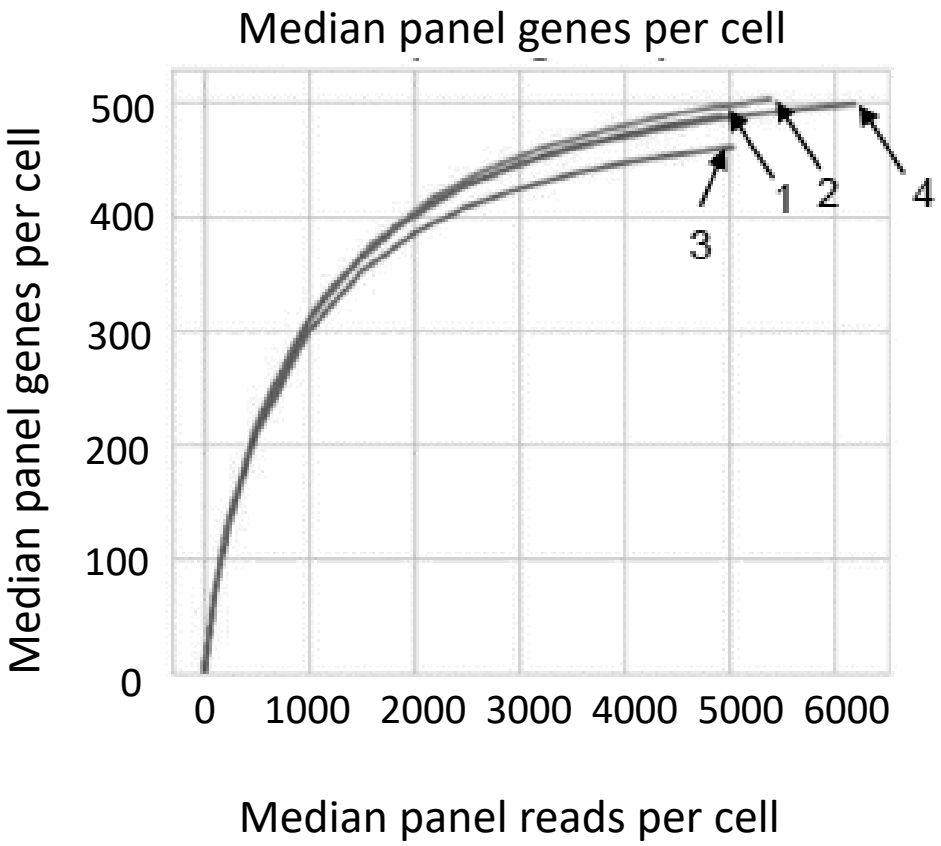

Median panel genes per cell
Median panel genes per cell
500
400
300
200
100
0
0
1000
2000
3000
4000
5000
6000
Median panel reads per cell
1
2
3
4

FIG. 25