

FIG. 1

Normalization Control				
Control	Reads	Expected Ratio	Actual	QC Pass/Fail
GalileoNormC:100	124	-	-	-
GalileoNormC:1000	1,108	10x	8.9x	Pass
GalileoNormC:10000	9,978	10x	9.0x	Pass
GalileoNormC:100000	101,390	10x	10.2x	Pass

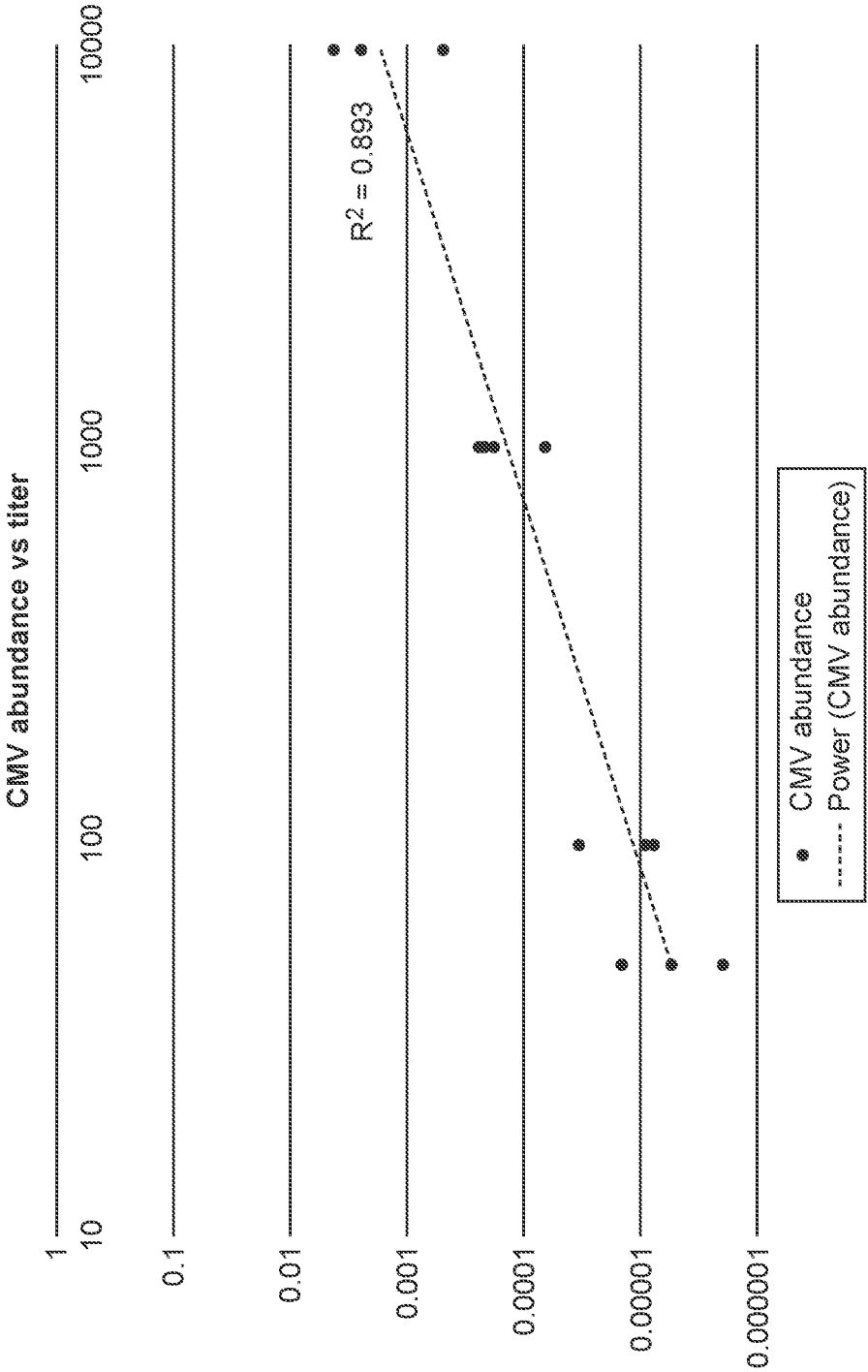


FIG. 2

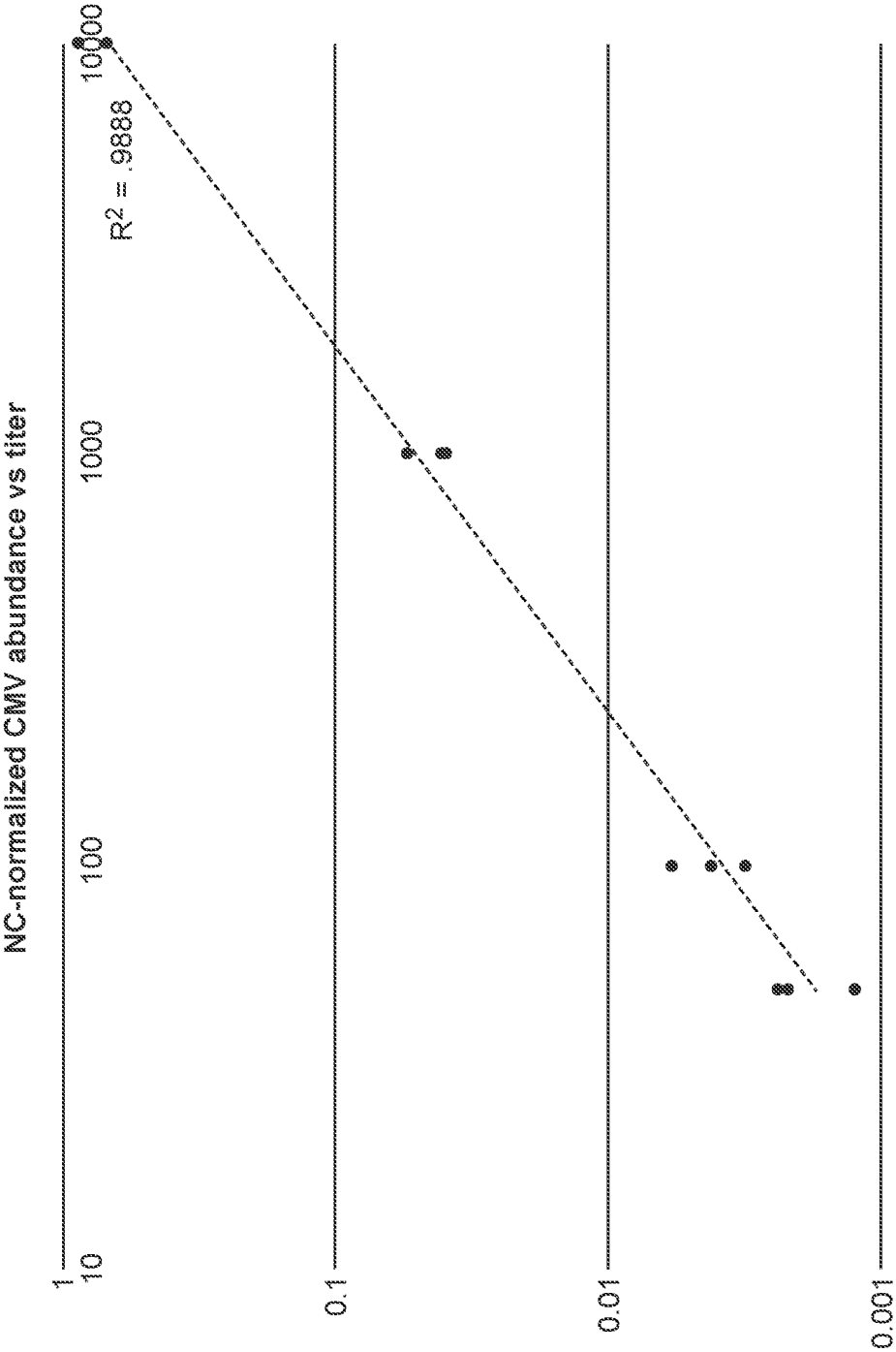


FIG. 3

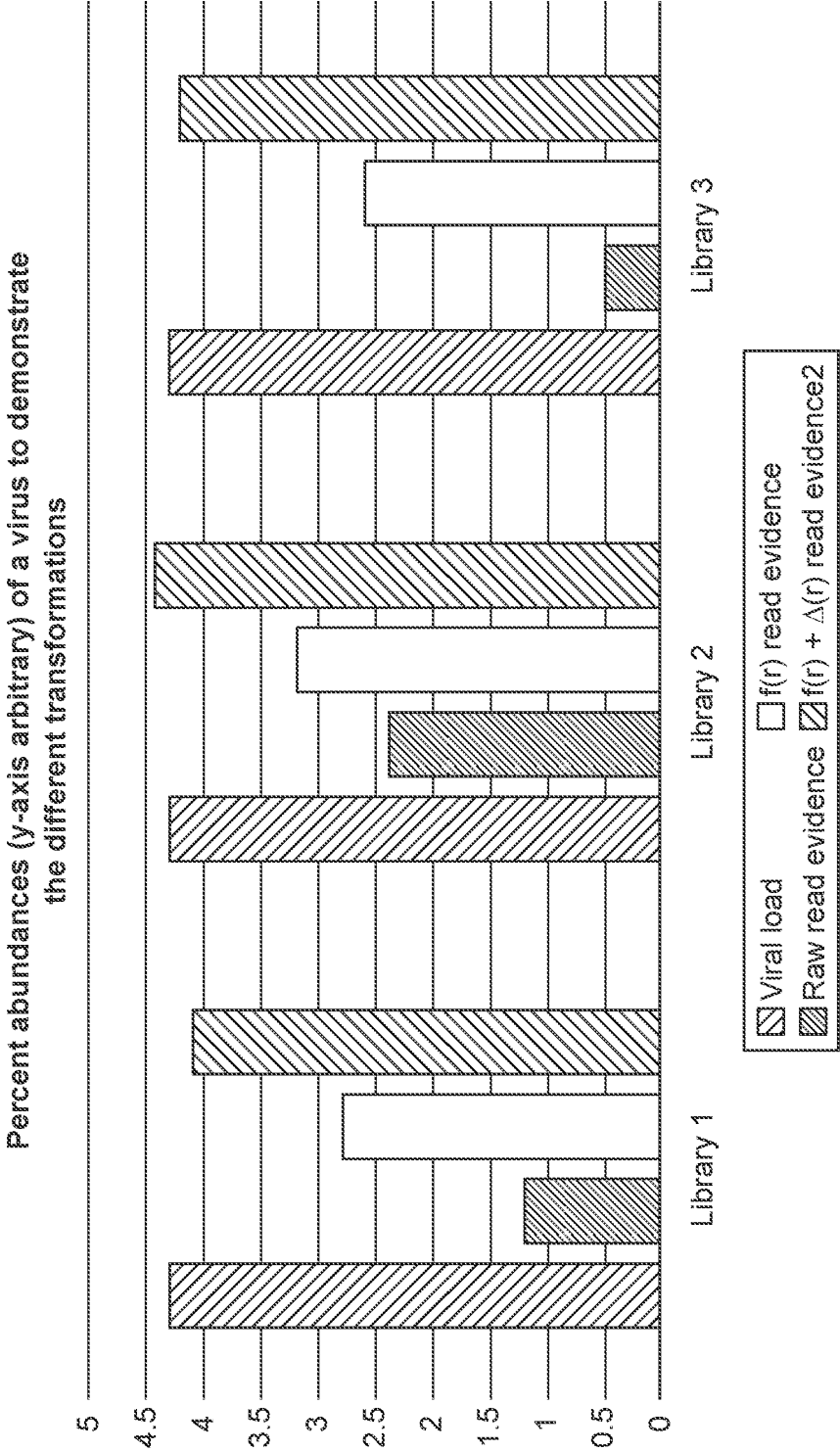


FIG. 4

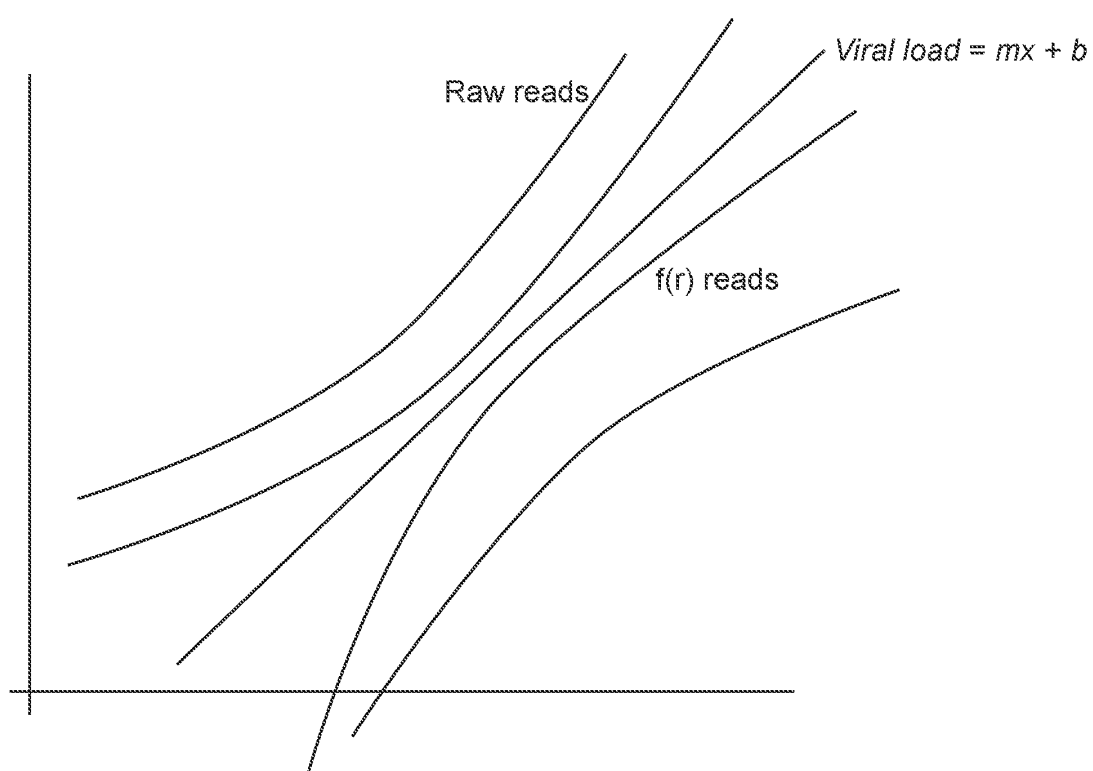


FIG. 5

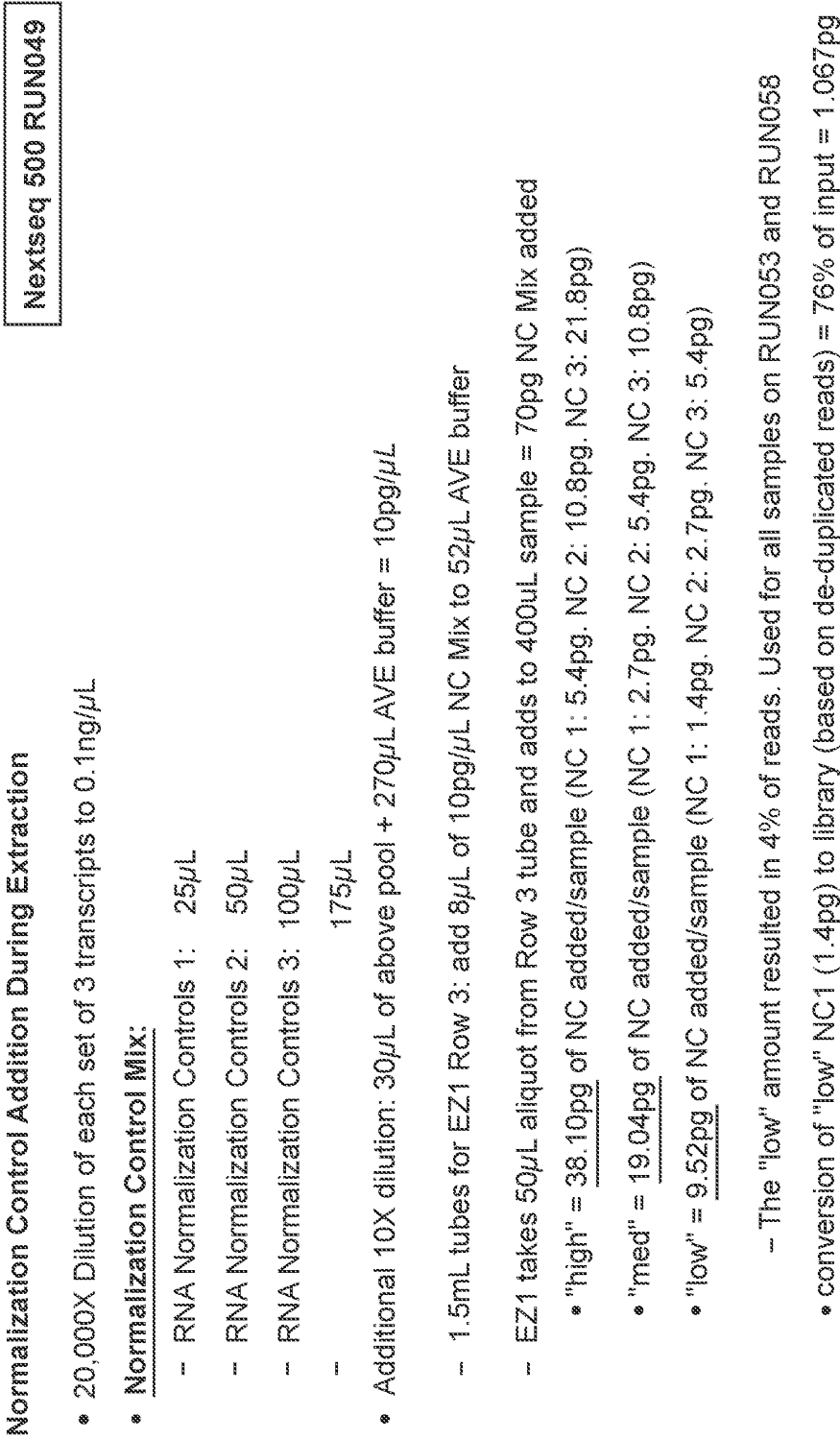


FIG. 6

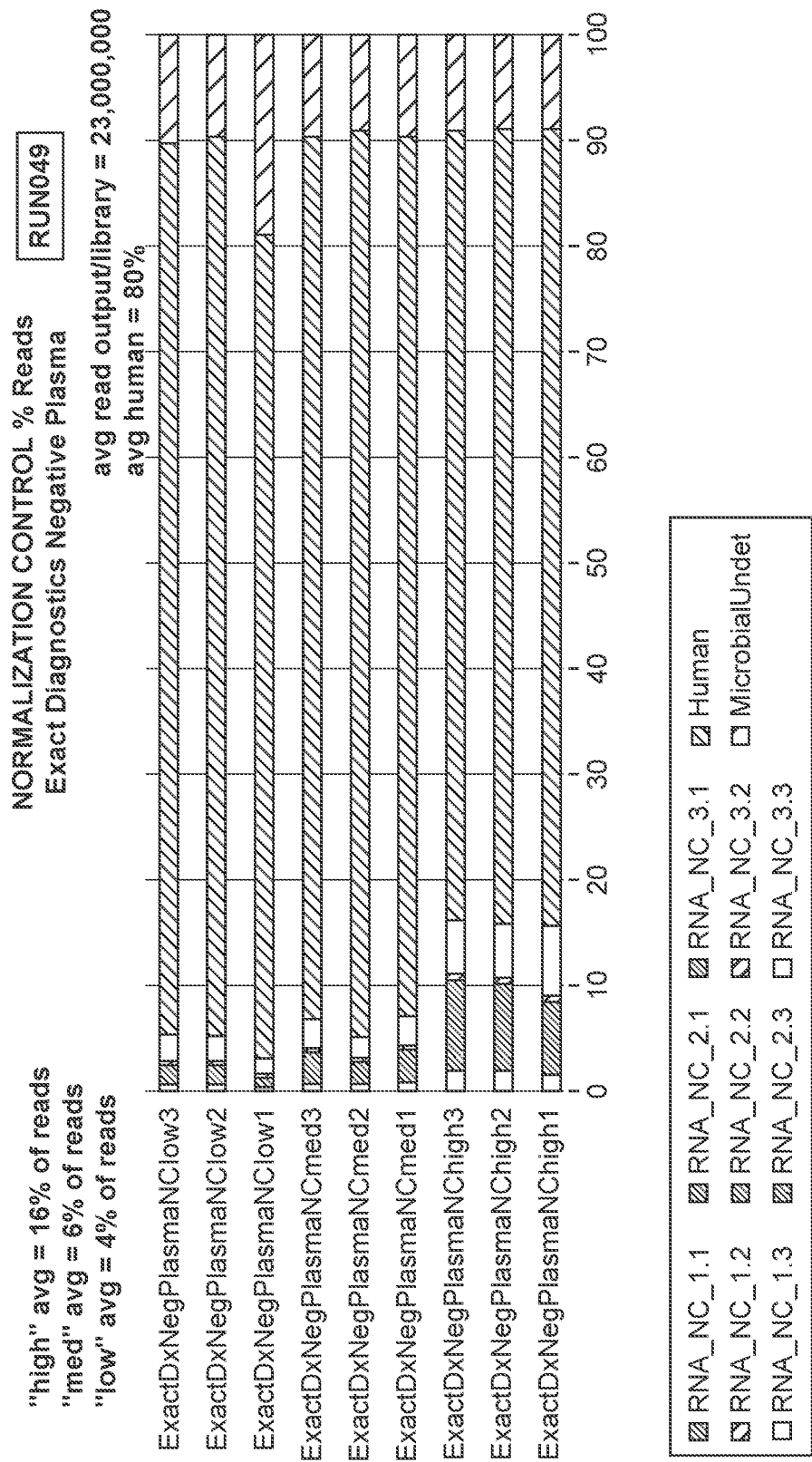


FIG. 7

[illegible]

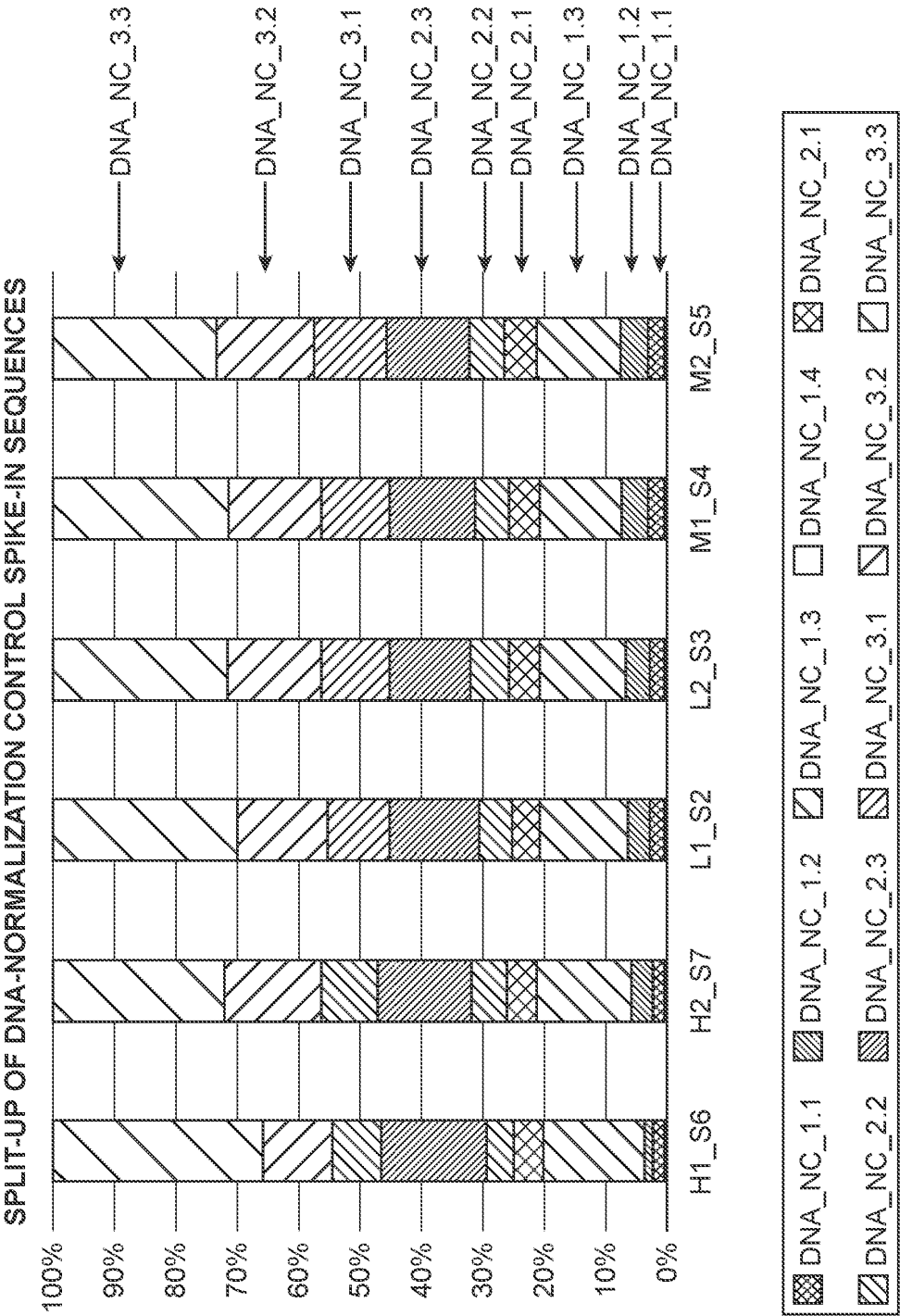


FIG. 9

Fasta Header	GC content	Total Count	Length
>Oligo177_RNA_Normalization_Controls_1_with_T7_RNA_polymerase_promoter_for_IVT	45.41284404	218	436
>Oligo178_RNA_Normalization_Controls_2_with_T7_RNA_polymerase_promoter_for_IVT	46.78899083	218	436
>Oligo179_RNA_Normalization_Controls_3_with_T7_RNA_polymerase_promoter_for_IVT	43.57798165	218	436
>Oligo180_RNA_Normalization_Controls_4_with_T7_RNA_polymerase_promoter_for_IVT	46.73913043	276	552
>Oligo181_RNA_Normalization_Controls_5_with_T7_RNA_polymerase_promoter_for_IVT	43.84057971	276	552
>Oligo182_RNA_Normalization_Control_6_with_T7_RNA_polymerase_promoter_for_IVT	40.57971014	276	552
>Oligo183_RNA_Normalization_Control_7_with_T7_RNA_polymerase_promoter_for_IVT	44.35318275	487	974
>Oligo184_RNA_Normalization_Control_8_with_T7_RNA_polymerase_promoter_for_IVT	43.32648871	487	974
>Oligo185_RNA_Normalization_Control_9_with_T7_RNA_polymerase_promoter_for_IVT	43.53182752	487	974
>Oligo186_RNA_Normalization_Control_1	49.77777778	225	450
>Oligo187_RNA_Normalization_Control_2	52	225	450
>Oligo188_RNA_Normalization_Control_3	49.77777778	225	450
>Oligo189_RNA_Normalization_Control_4	42.15686275	306	612
>Oligo190_RNA_Normalization_Control_5	43.46405229	306	612
>Oligo191_RNA_Normalization_Control_6	41.50326797	306	612
>Oligo192_RNA_Normalization_Control_7	45.64796905	517	1034
>Oligo193_RNA_Normalization_Control_8	46.61508704	517	1034
>Oligo194_RNA_Normalization_Control_9	44.87427466	517	1034
>Oligo241_manorm1seg1	47.05882353	255	510
>Oligo242_manorm2seg1	48.62745098	255	510
>Oligo243_manorm3seg1	53.7254902	255	510
>Oligo244_manorm1seg2	51.7571885	313	626
>Oligo245_manorm2seg2	51.7571885	313	626
>Oligo246_manorm3seg2	44.7284345	313	626
>Oligo247_manorm2seg3	50.57251908	524	1048
>Oligo248_manorm3seg3	49.42748092	524	1048
>Oligo249_manorm4seg3	48.85496183	524	1048
>Oligo250_dnanorm1seg1	46.66666667	225	450
>Oligo251_dnanorm2seg1	55.55555556	225	450
>Oligo252_dnanorm3seg1	54.22222222	225	450
>Oligo253_dnanorm1seg2	49.34640523	306	612
>Oligo254_dnanorm2seg2	50.65359477	306	612
>Oligo255_dnanorm3seg2	49.01960784	306	612
>Oligo256_dnanorm1seg3	49.9032882	517	1034
>Oligo257_dnanorm2seg3	48.54932302	517	1034
>Oligo258_dnanorm3seg3	48.54932302	517	1034

FIG. 10

Oligo number	Final Conc ng/ul	Ratio to total amt of DNA
Oligo186	4.20E-03	0.420
Oligo187	4.21E-04	0.042
Oligo188	2.11E-04	0.021
Oligo189	2.86E-03	0.286
Oligo190	4.29E-04	0.043
Oligo191	1.43E-04	0.014
Oligo192	9.68E-04	0.097
Oligo193	4.84E-04	0.048
Oligo194	9.68E-05	0.010
Oligo250	4.22E-05	0.004
Oligo251	2.11E-05	0.002
Oligo253	4.31E-05	0.004
Oligo254	1.43E-05	0.001
Oligo256	4.84E-05	0.005
Oligo257	9.71E-06	0.001
Lib Size		

FIG. 11

FIG. 12

Group	Length (kb)	Length (bp)	Number of Molecules	Total Attomoles/Fragment	Concentration (attomoles/ μ L)
RNA_NC_1.1 A	0.175	175	263158	0.44	0.07
RNA_NC_1.2 B	0.25	250	1144737	1.90	0.29
RNA_NC_1.3 C	0.45	450	5131579	8.52	1.28
RNA_NC_2.1 A	0.175	175	2789474	4.63	0.69
RNA_NC_2.2 B	0.25	250	4513157	7.49	1.12
RNA_NC_2.3 C	0.45	450	44473684	73.84	11.08
RNA_NC_3.1 A	0.175	175	4605263	7.65	1.15
RNA_NC_3.2 B	0.25	250	17500000	29.06	4.36
RNA_NC_3.3 C	0.45	450	77894737	129.33	19.40

FIG. 13

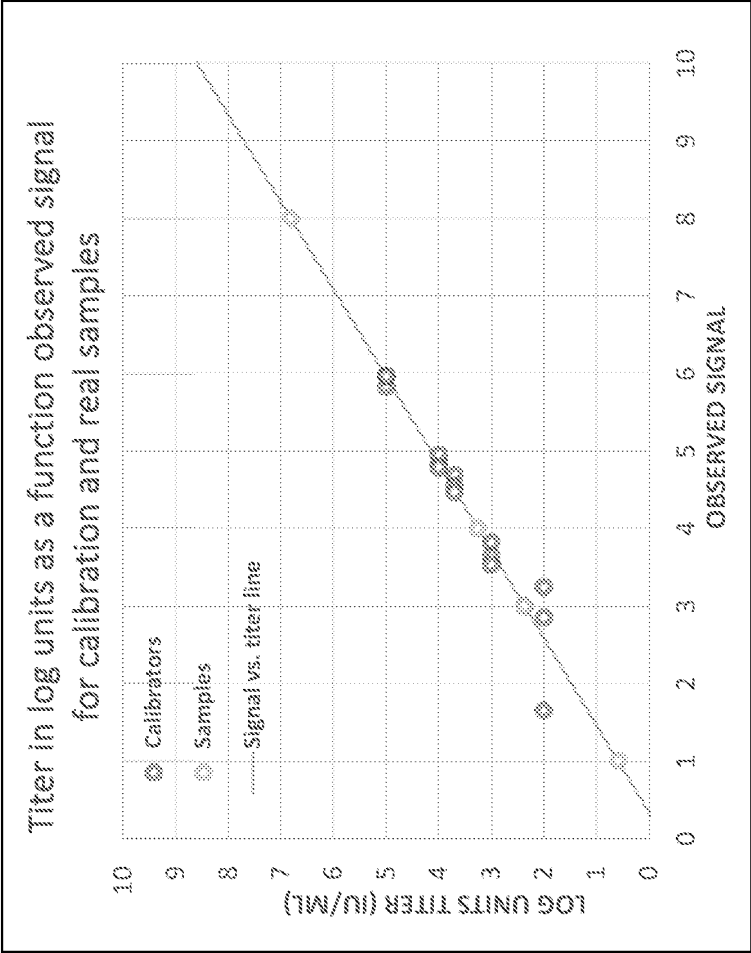
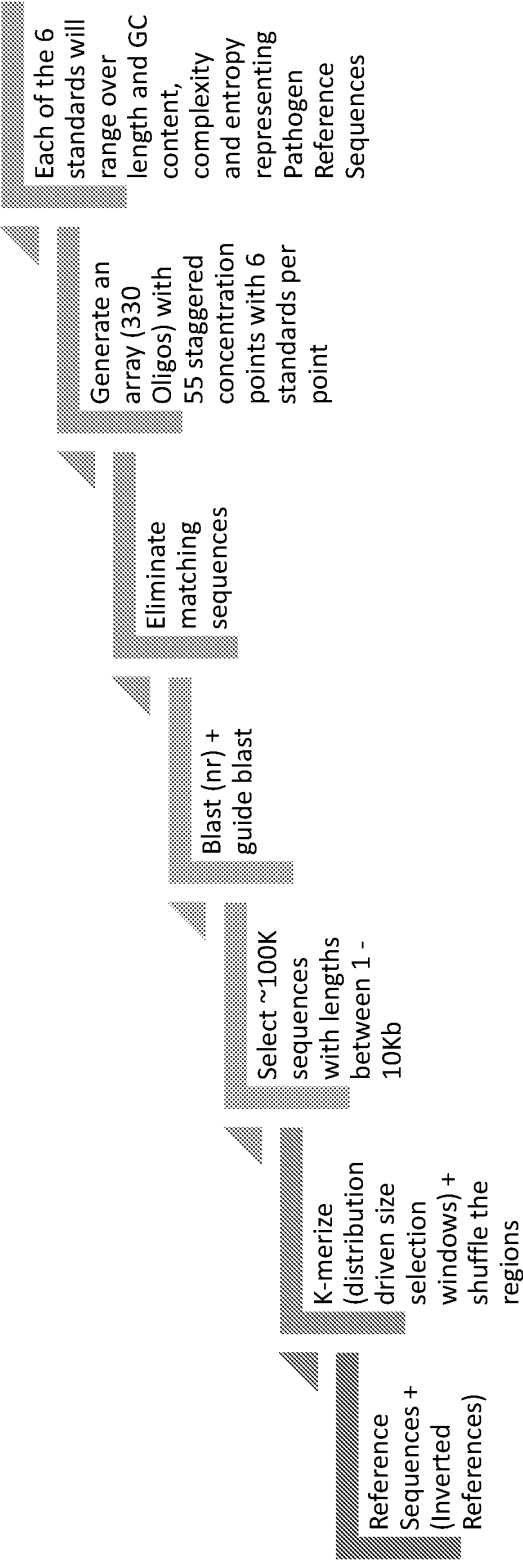


FIG. 14A
Example Normalization Control Design Pipeline



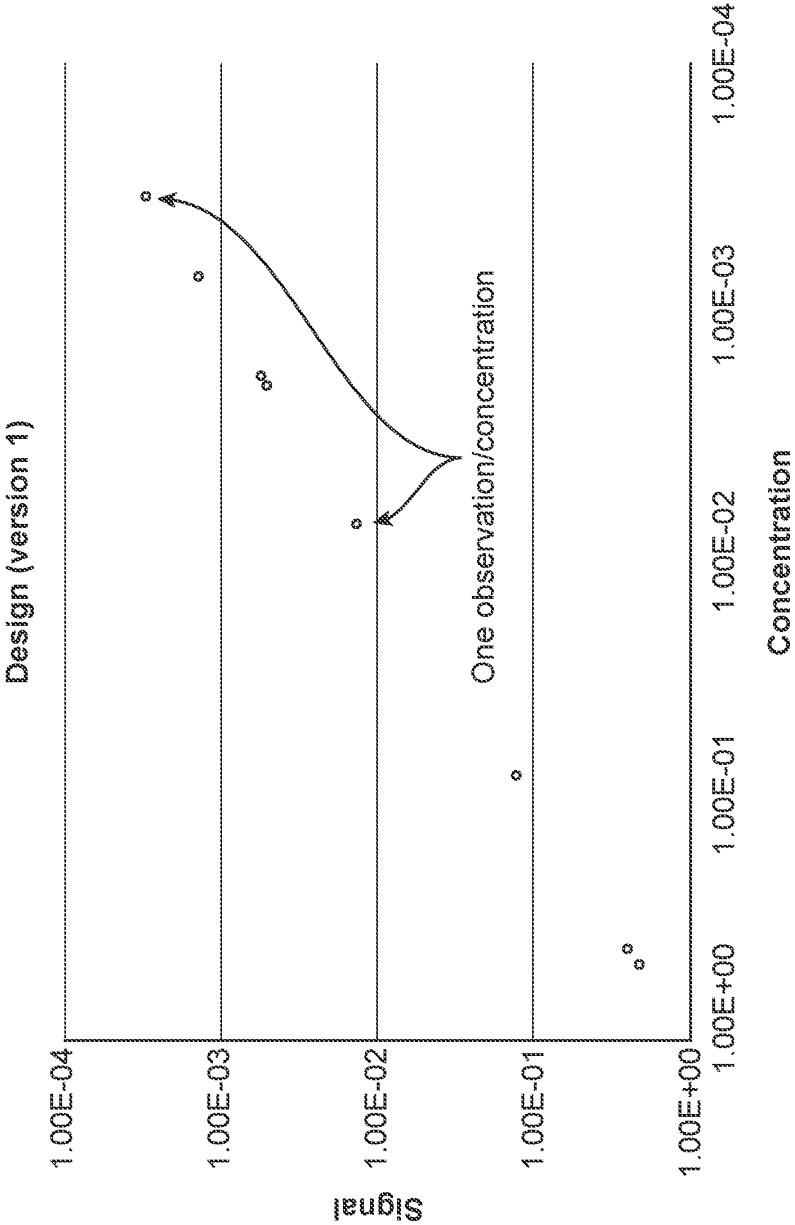


FIG. 14B

FIG. 14C

Name	Library		conc. In		Bottom Out (conc. Out
Oligo298.dna		298	4.88E-01	No	4.88E-01
Oligo299.dna		299	4.19E-01	No	4.19E-01
Oligo301.dna		301	8.13E-02	No	8.13E-02
Oligo302.dna		302	2.04E-03	No	2.04E-03
Oligo303.dna		303	7.63E-03	No	7.63E-03
Oligo304.dna		304	1.87E-03	No	1.87E-03
Oligo305.dna		305	7.39E-04	Bottoms out	7.39E-04
Oligo306.dna		306	3.45E-04	Bottoms out	3.45E-04

Design and Concentration (version 2)

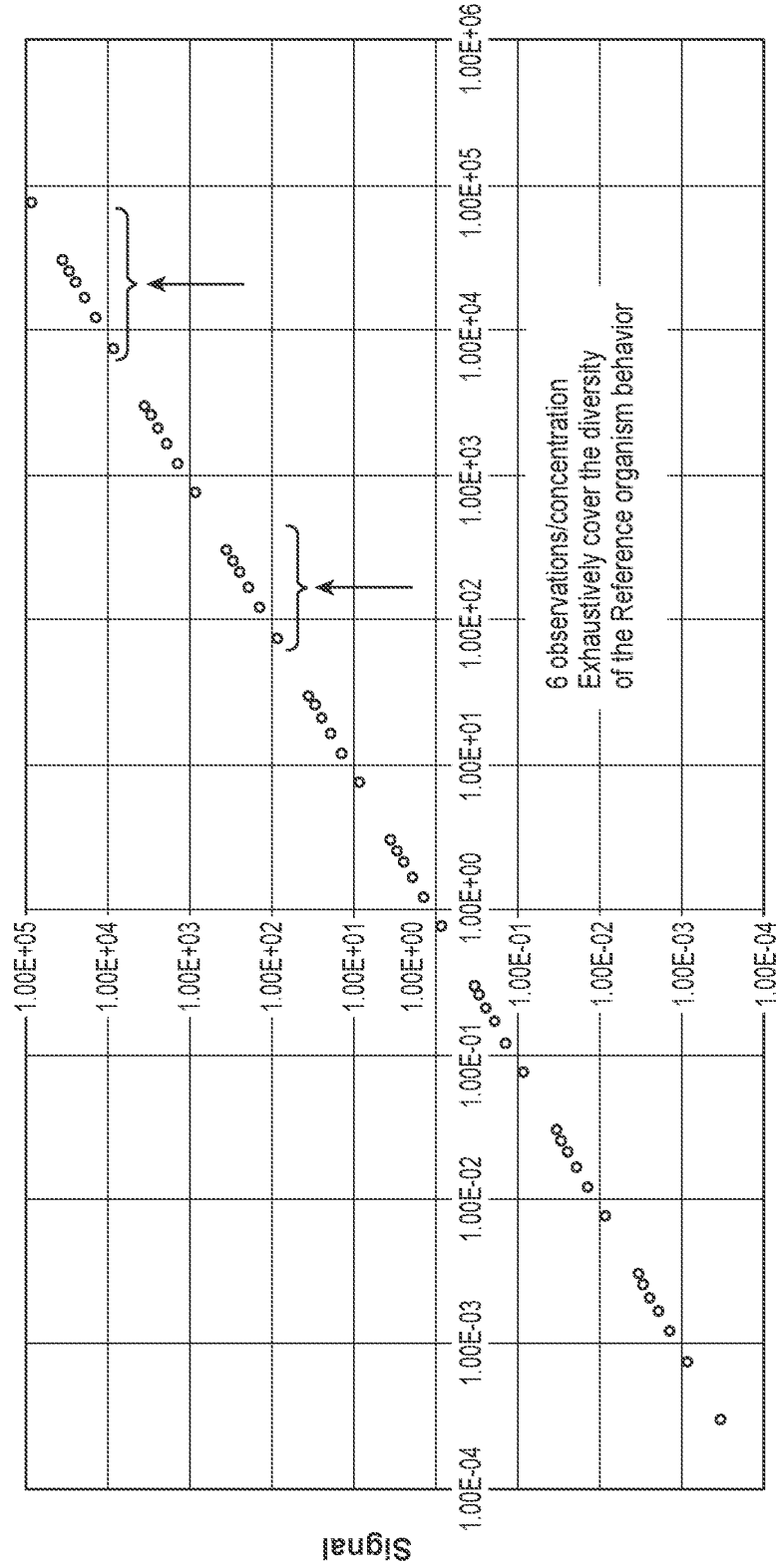
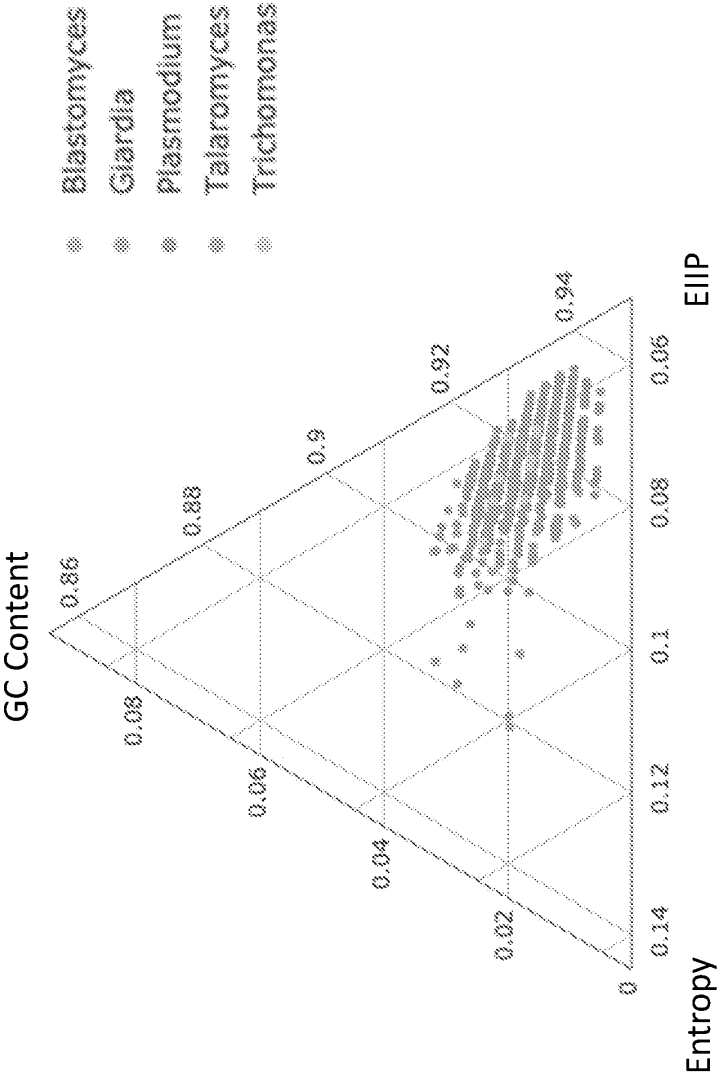


FIG. 14D

FIG. 15



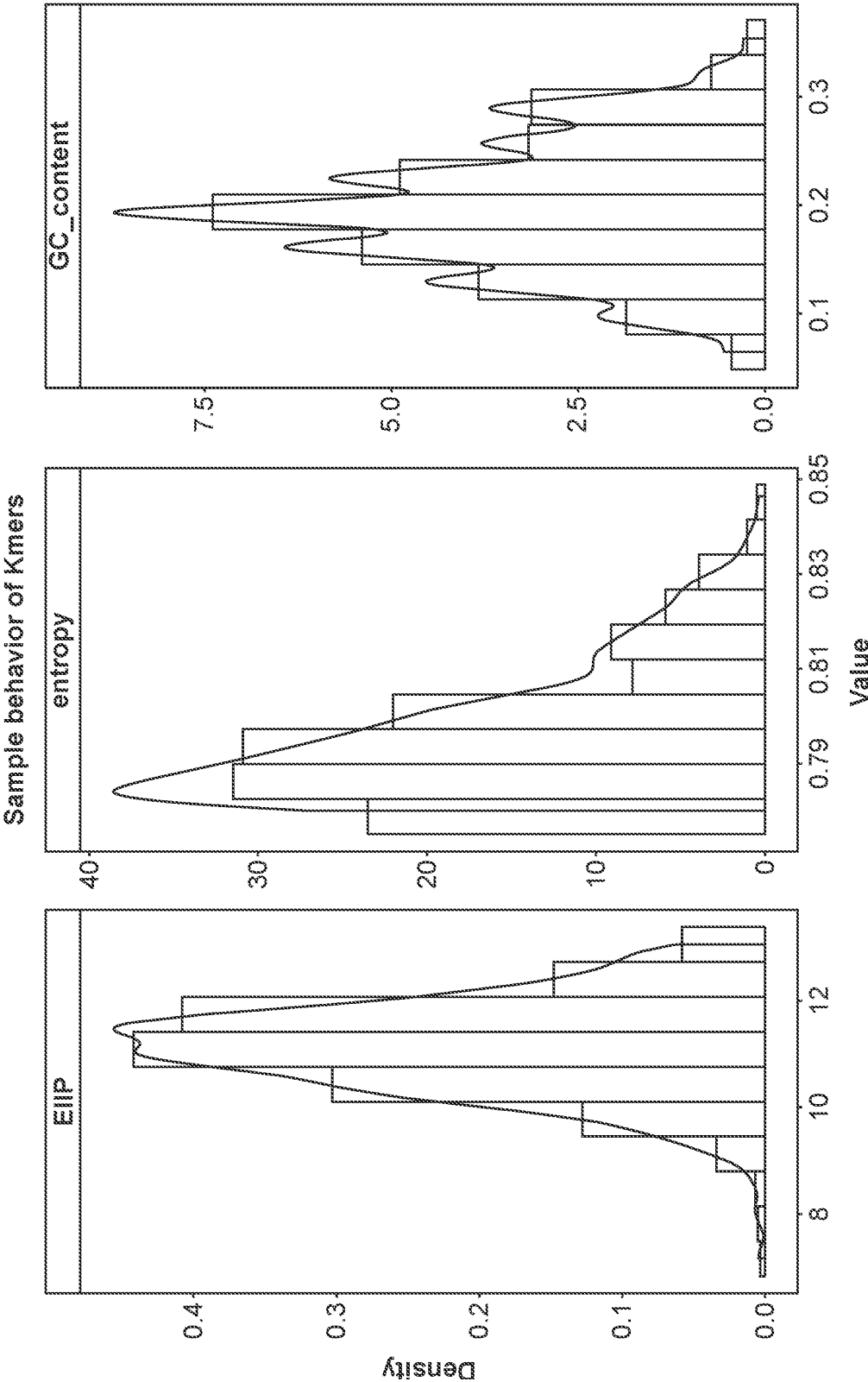


FIG. 16

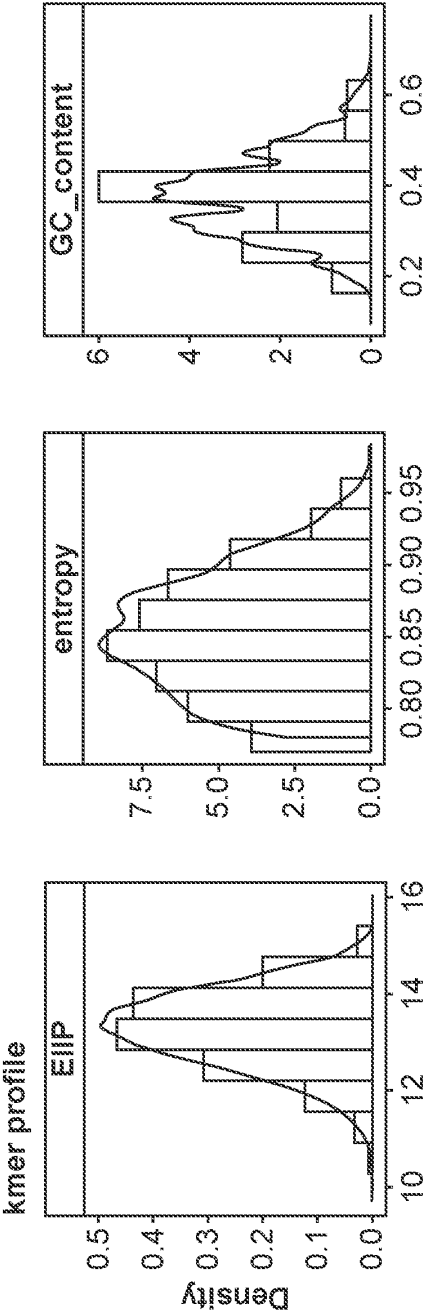


FIG. 17A

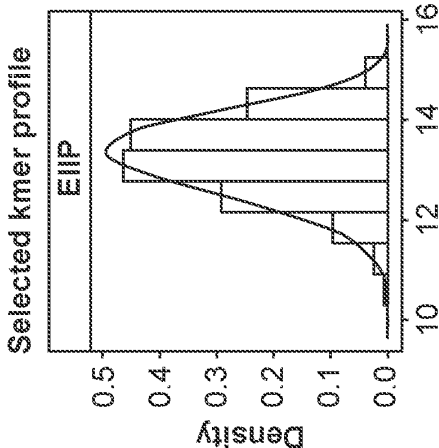


FIG. 17B

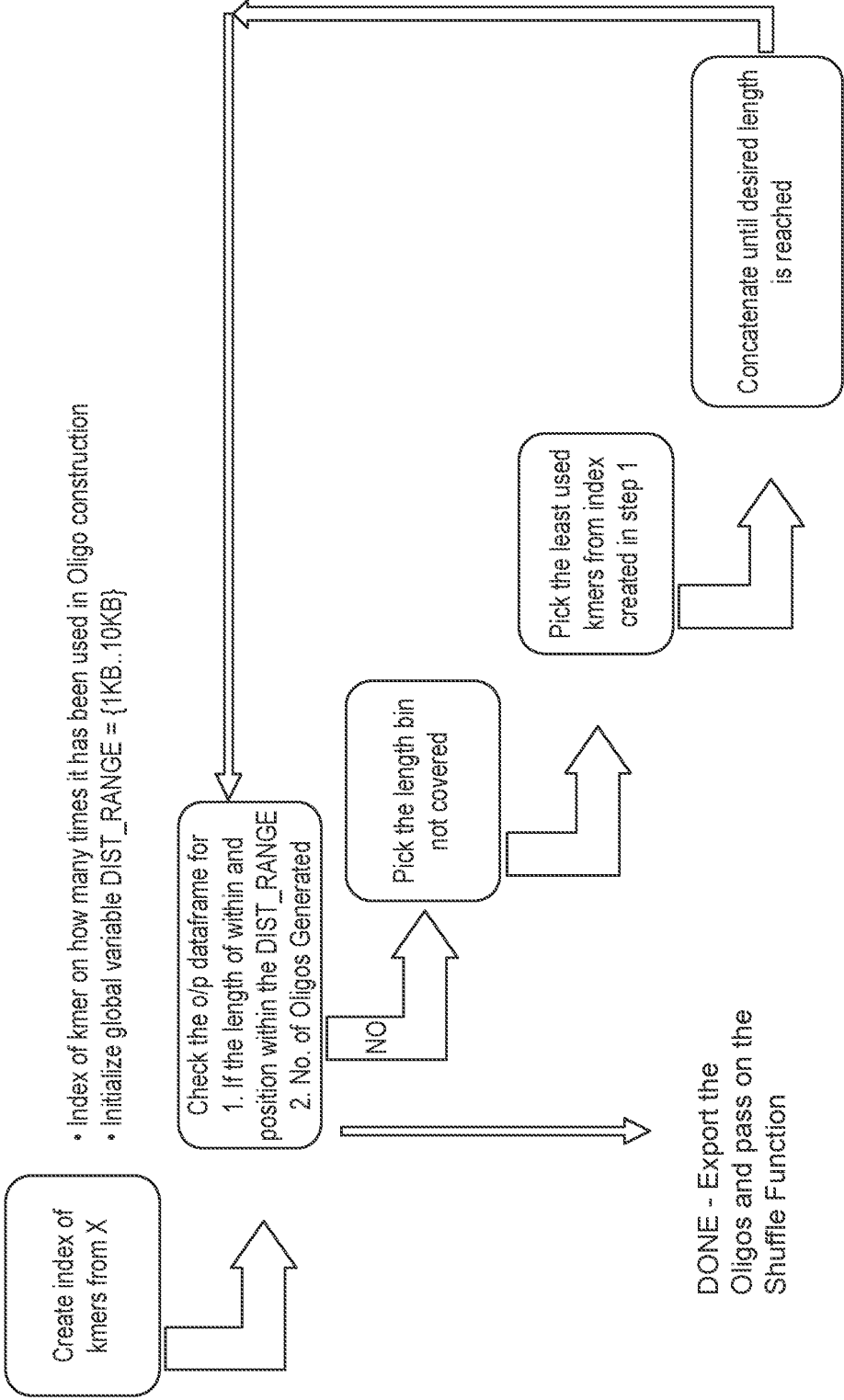


FIG. 18

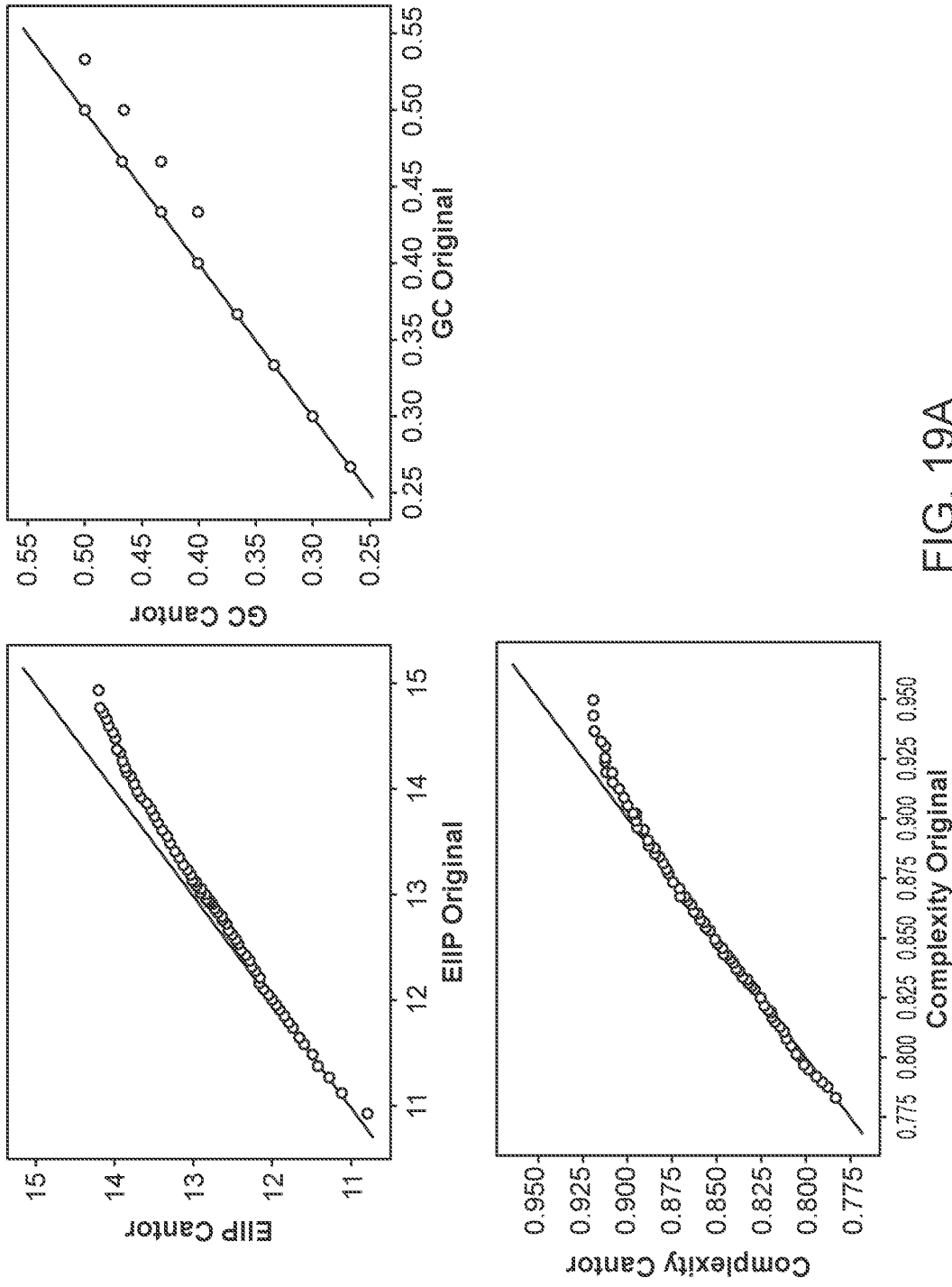
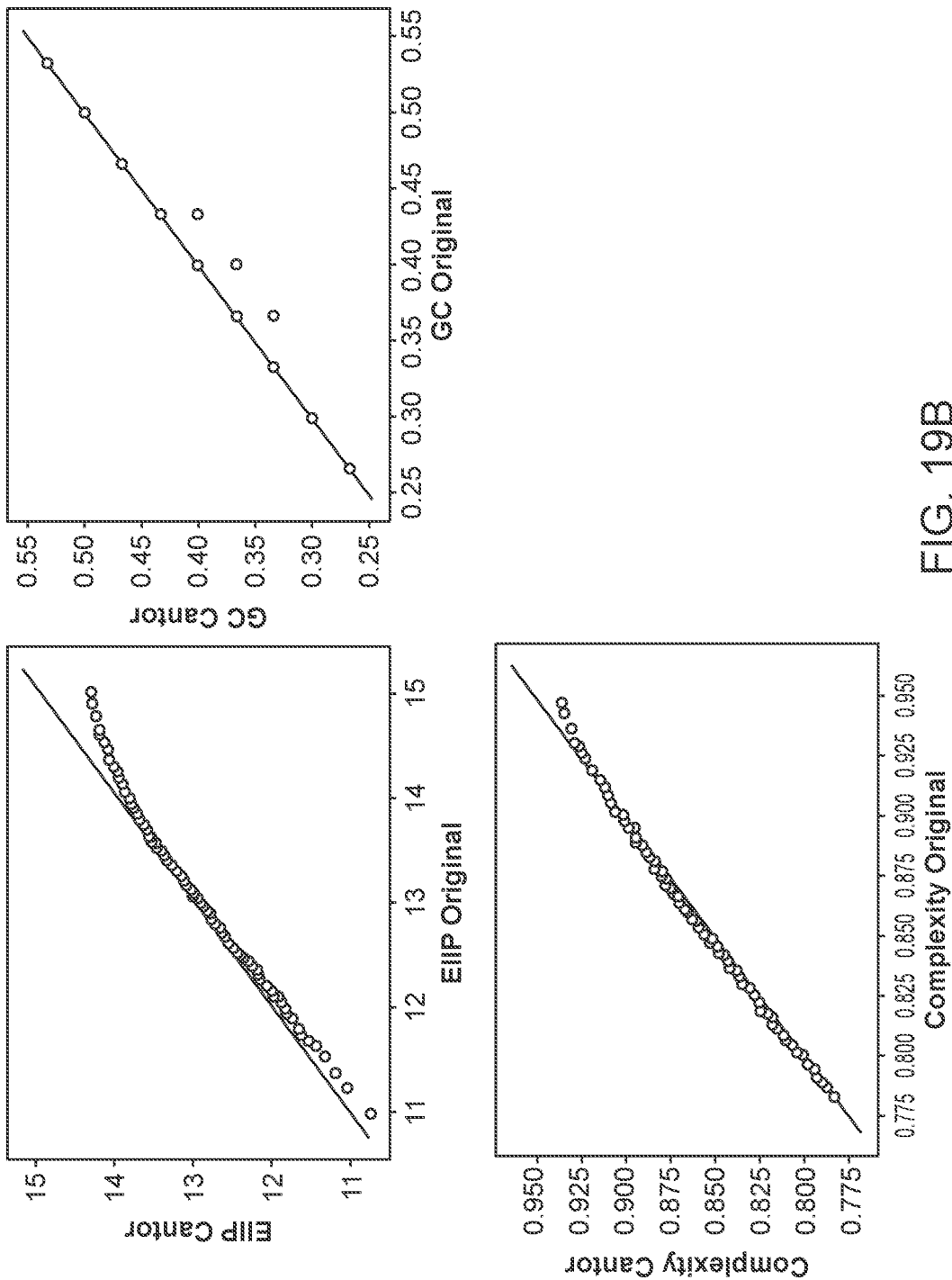


FIG. 19A



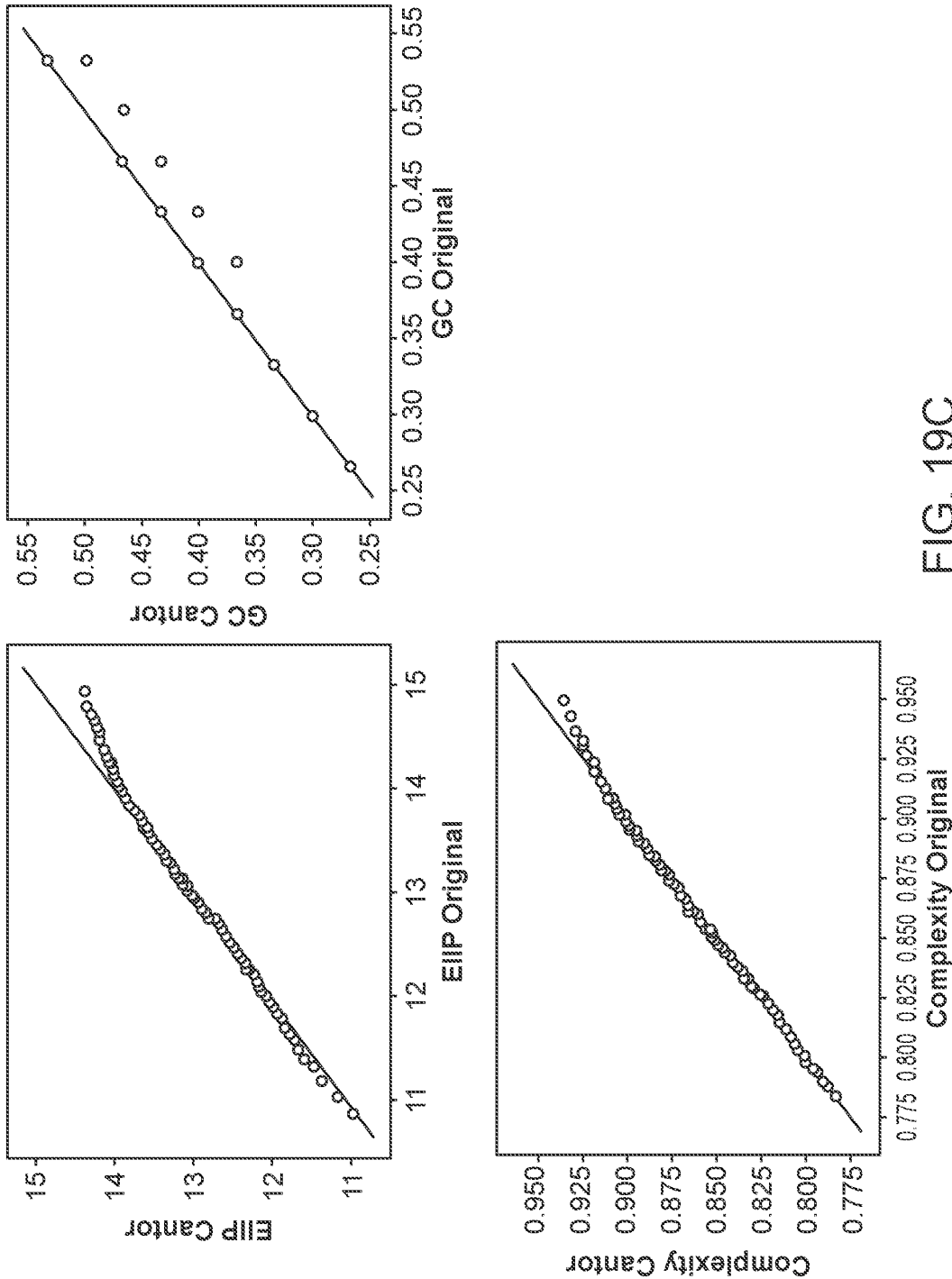
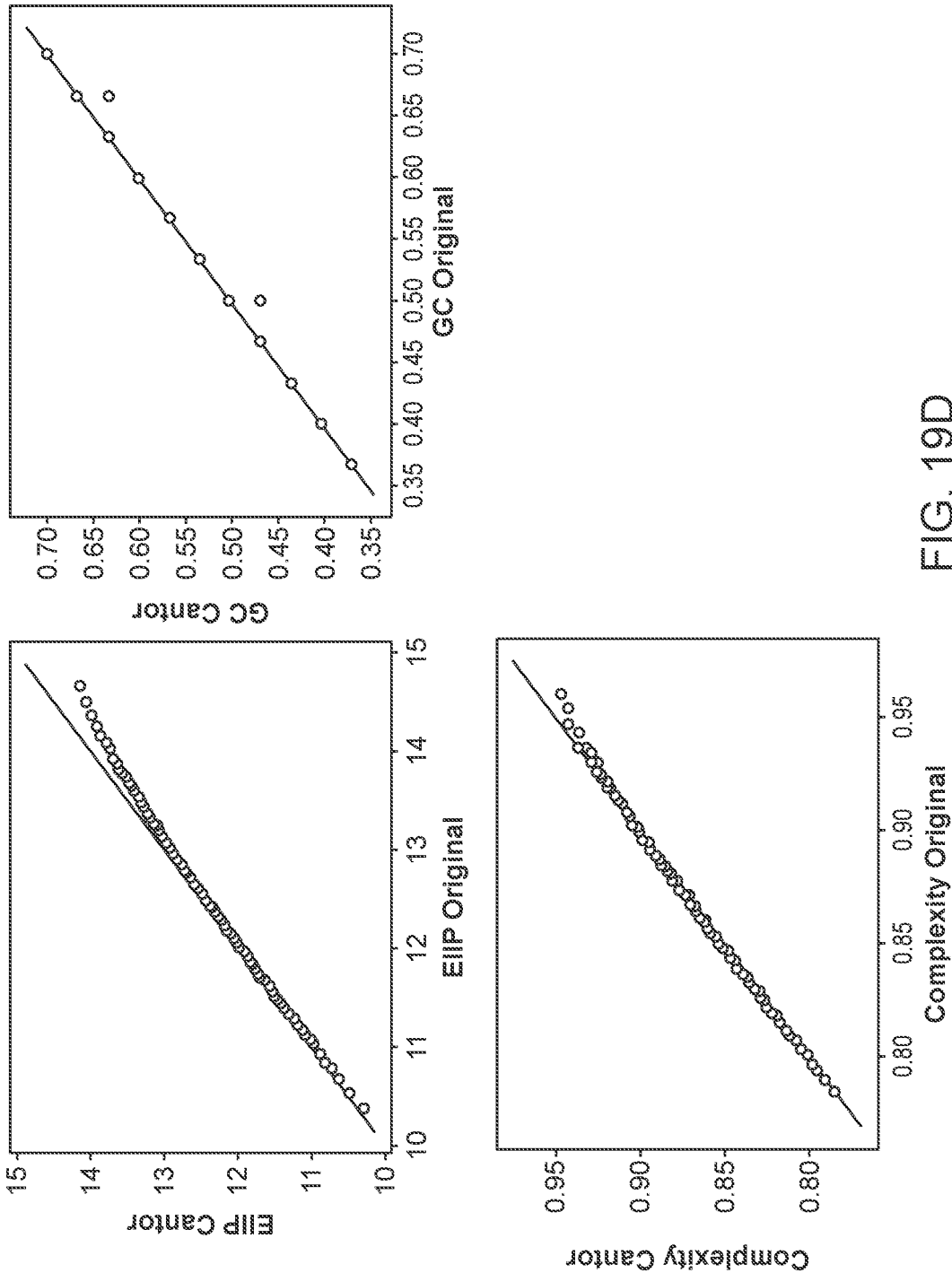


FIG. 19C



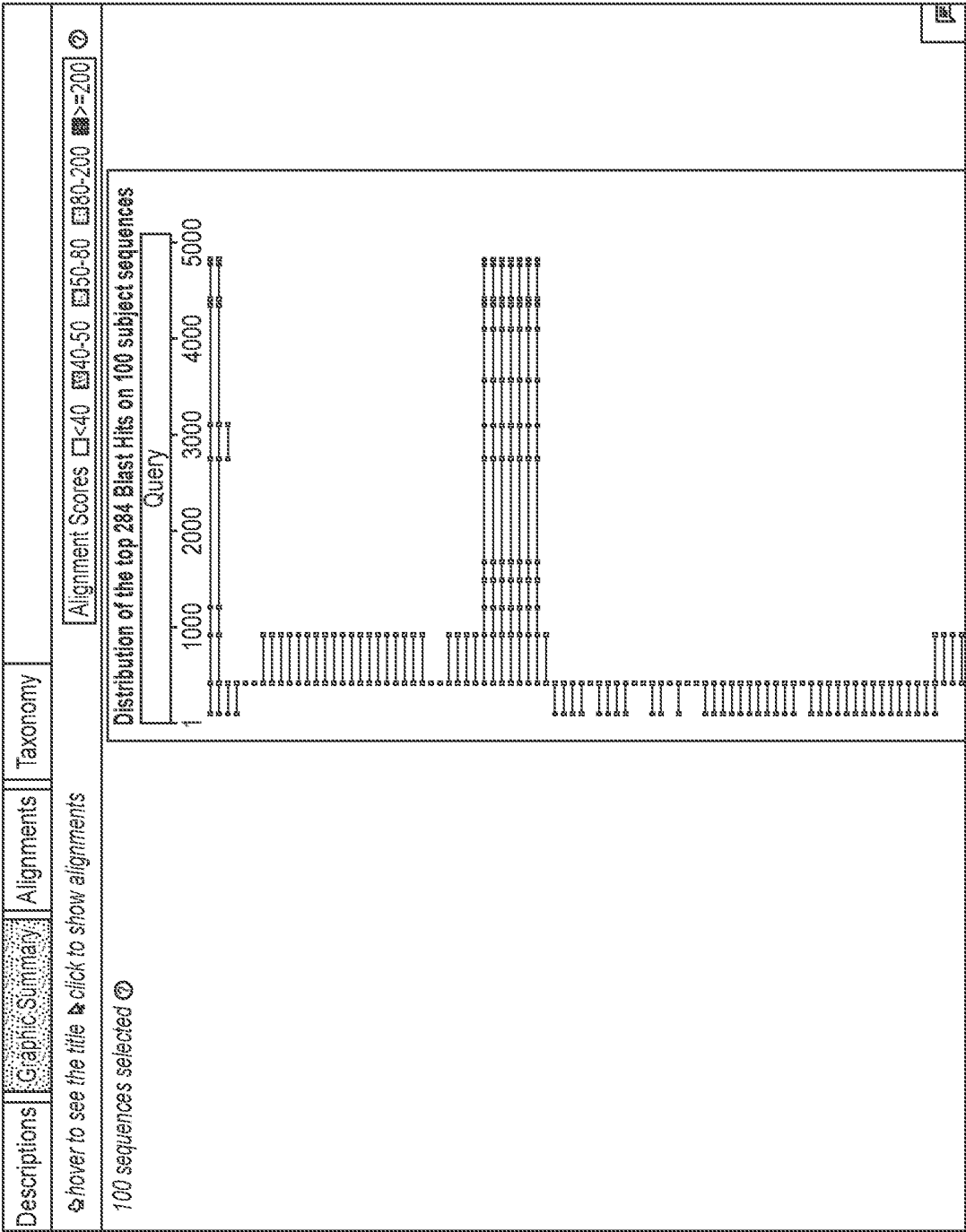


FIG. 20A

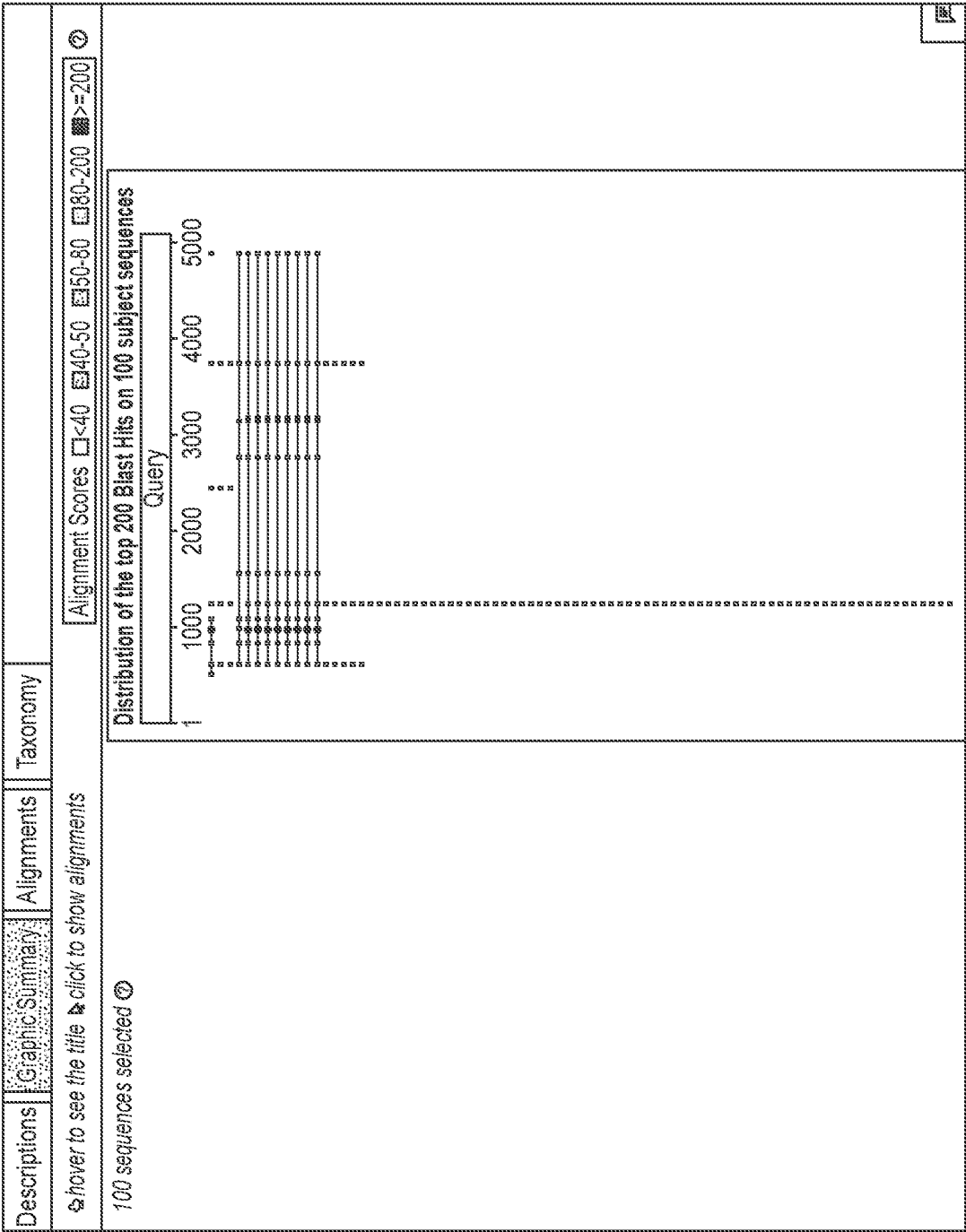


FIG. 20B

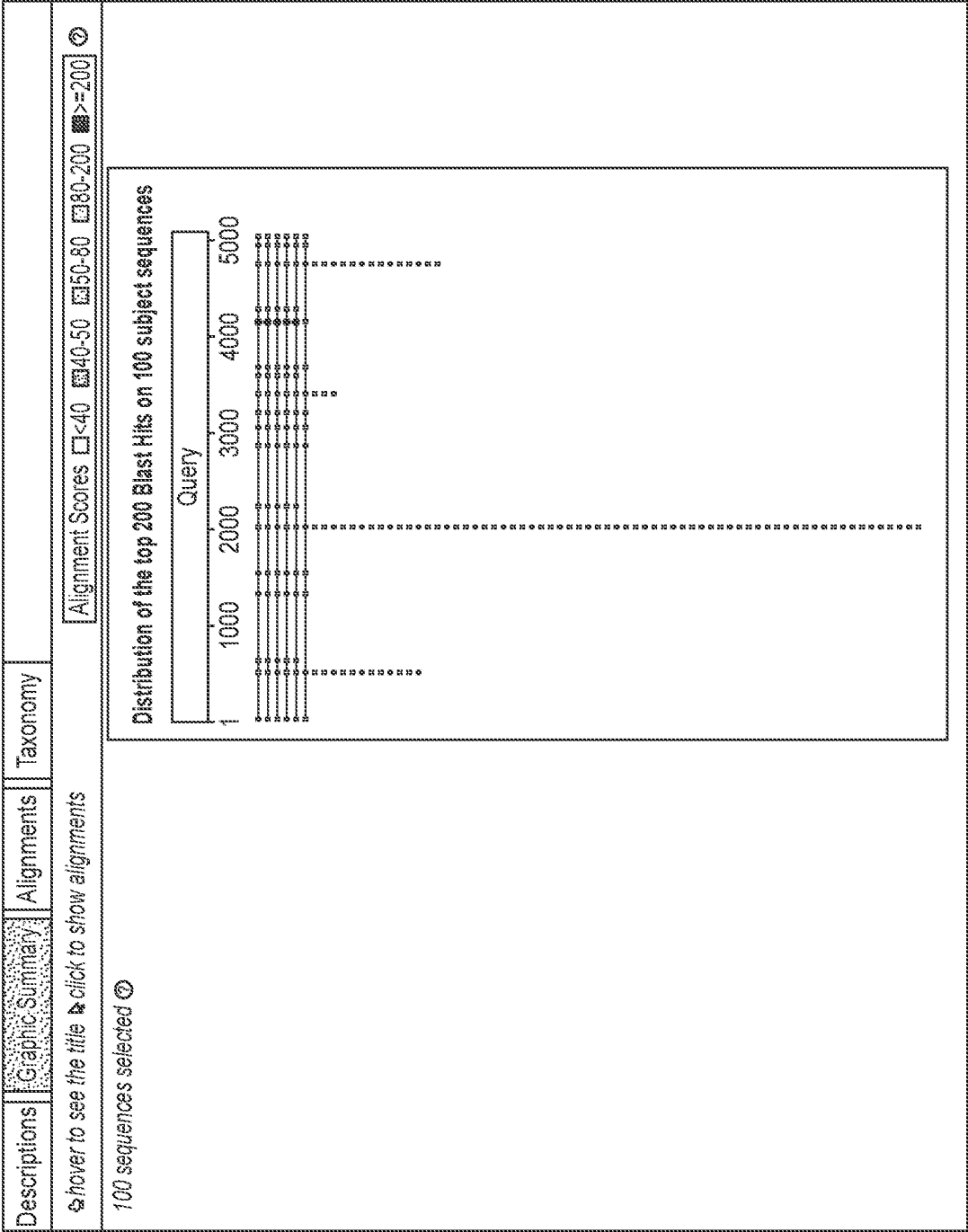


FIG. 20C

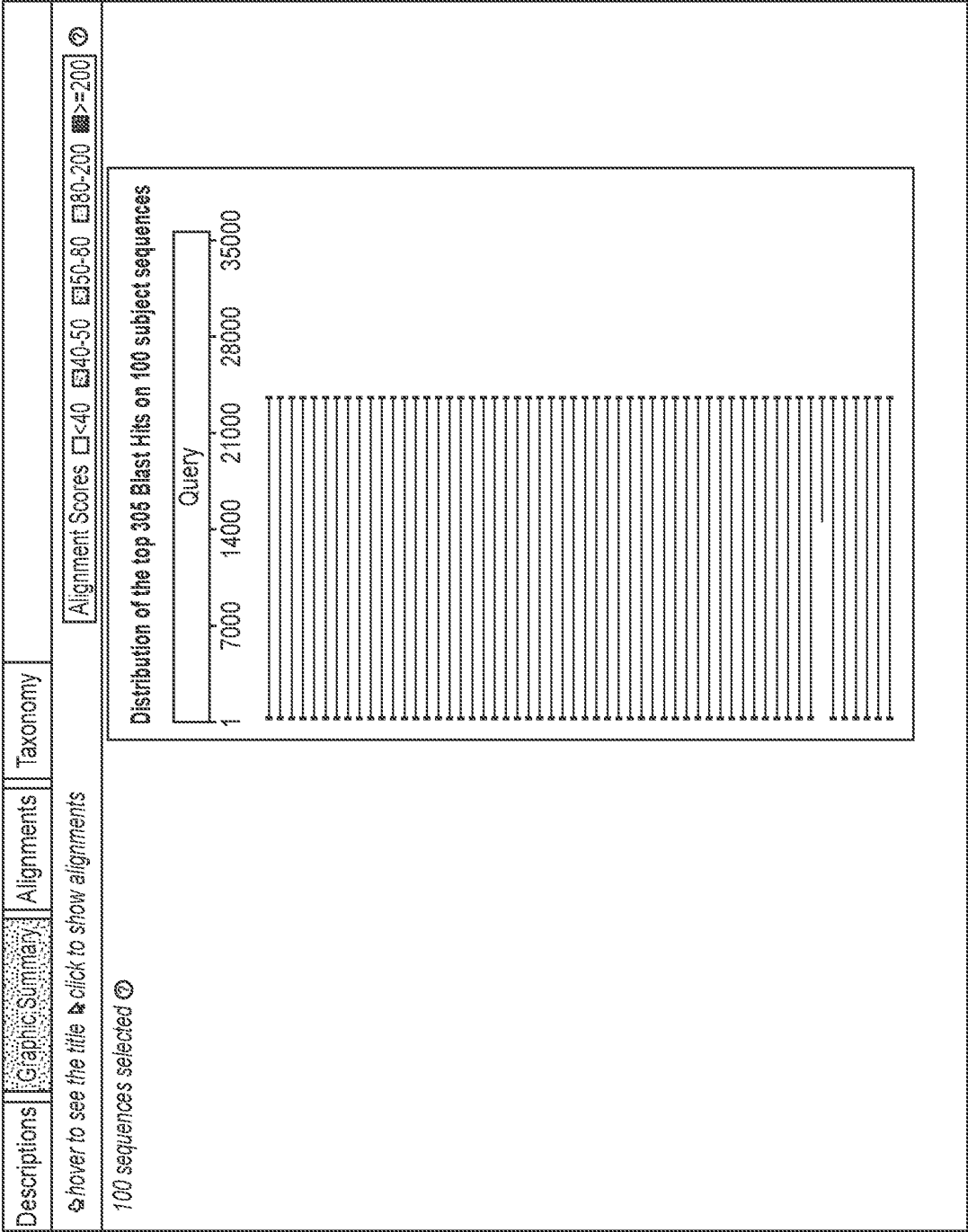


FIG. 20D

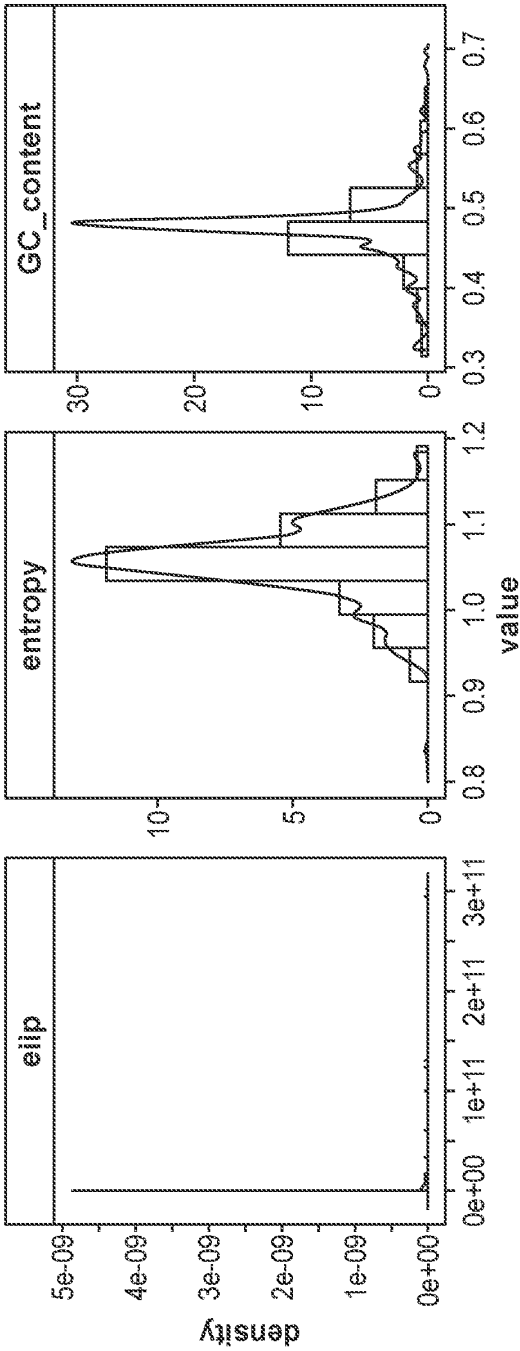


FIG. 21A

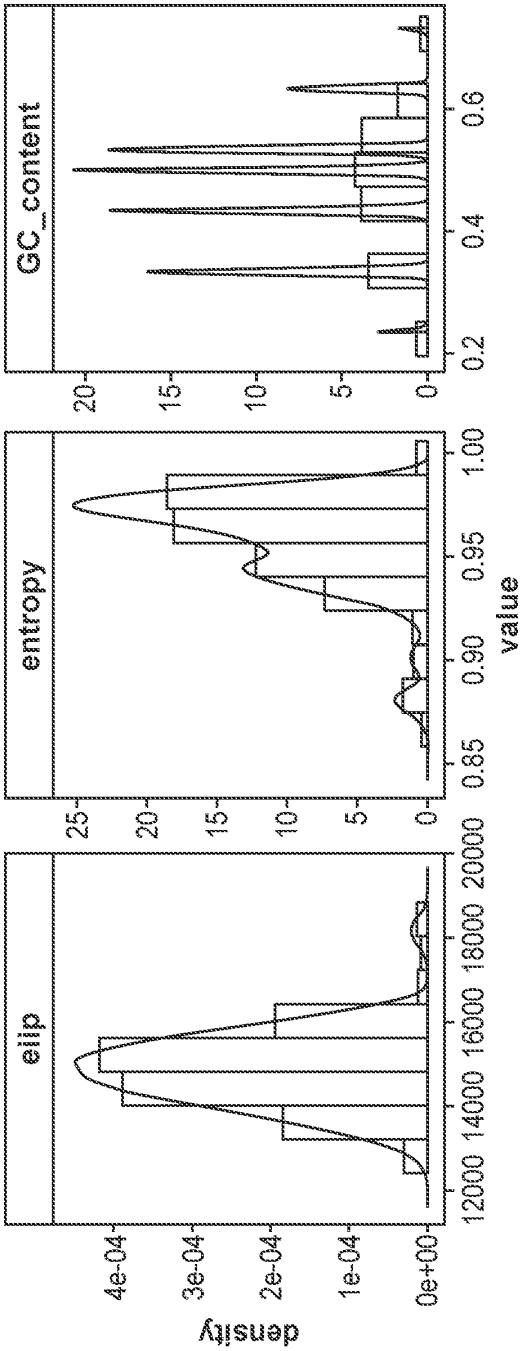


FIG. 21B

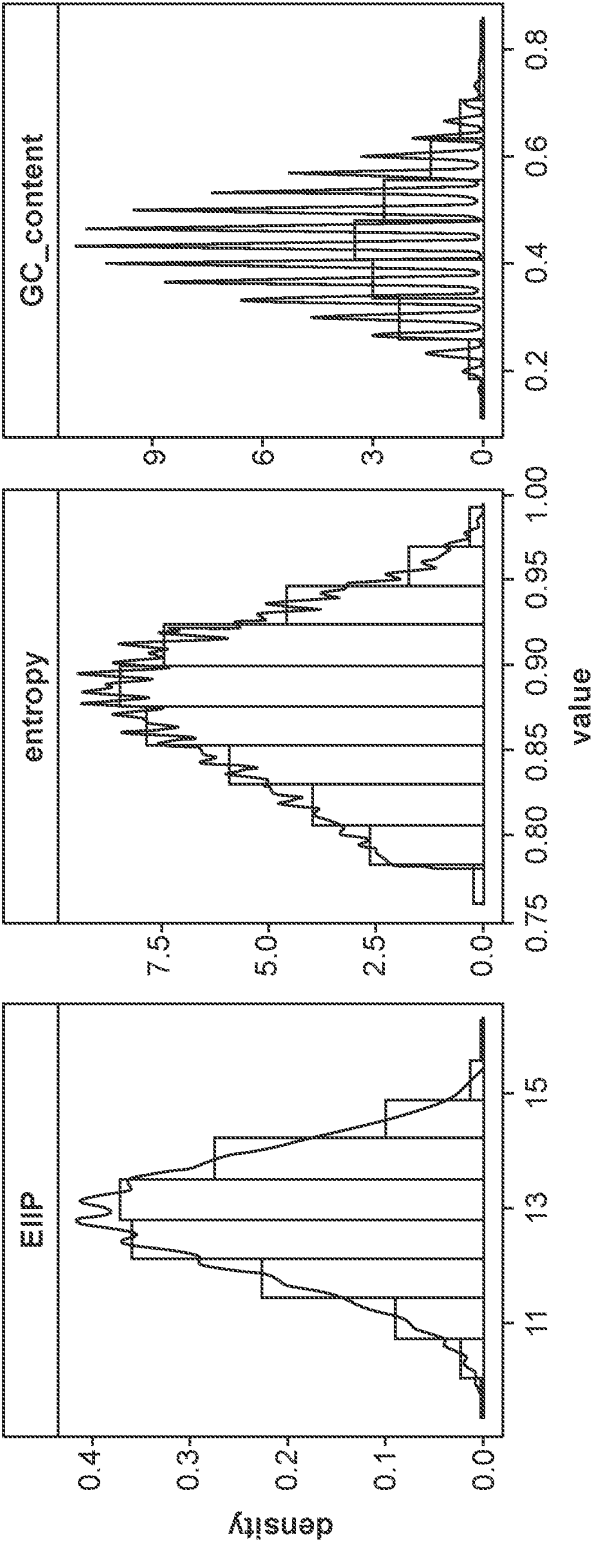
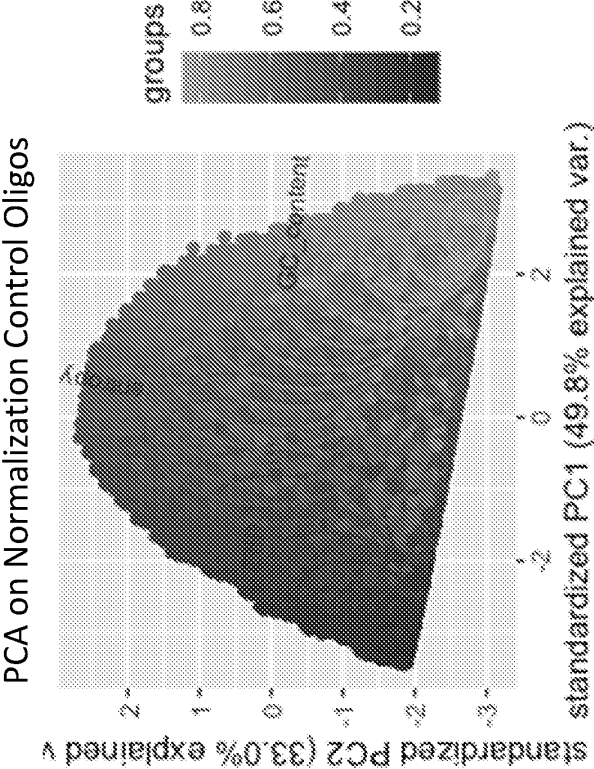
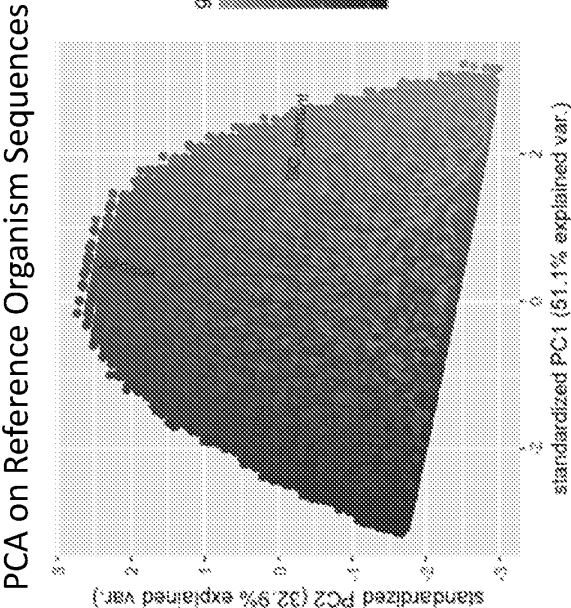


FIG. 21C

FIG. 22



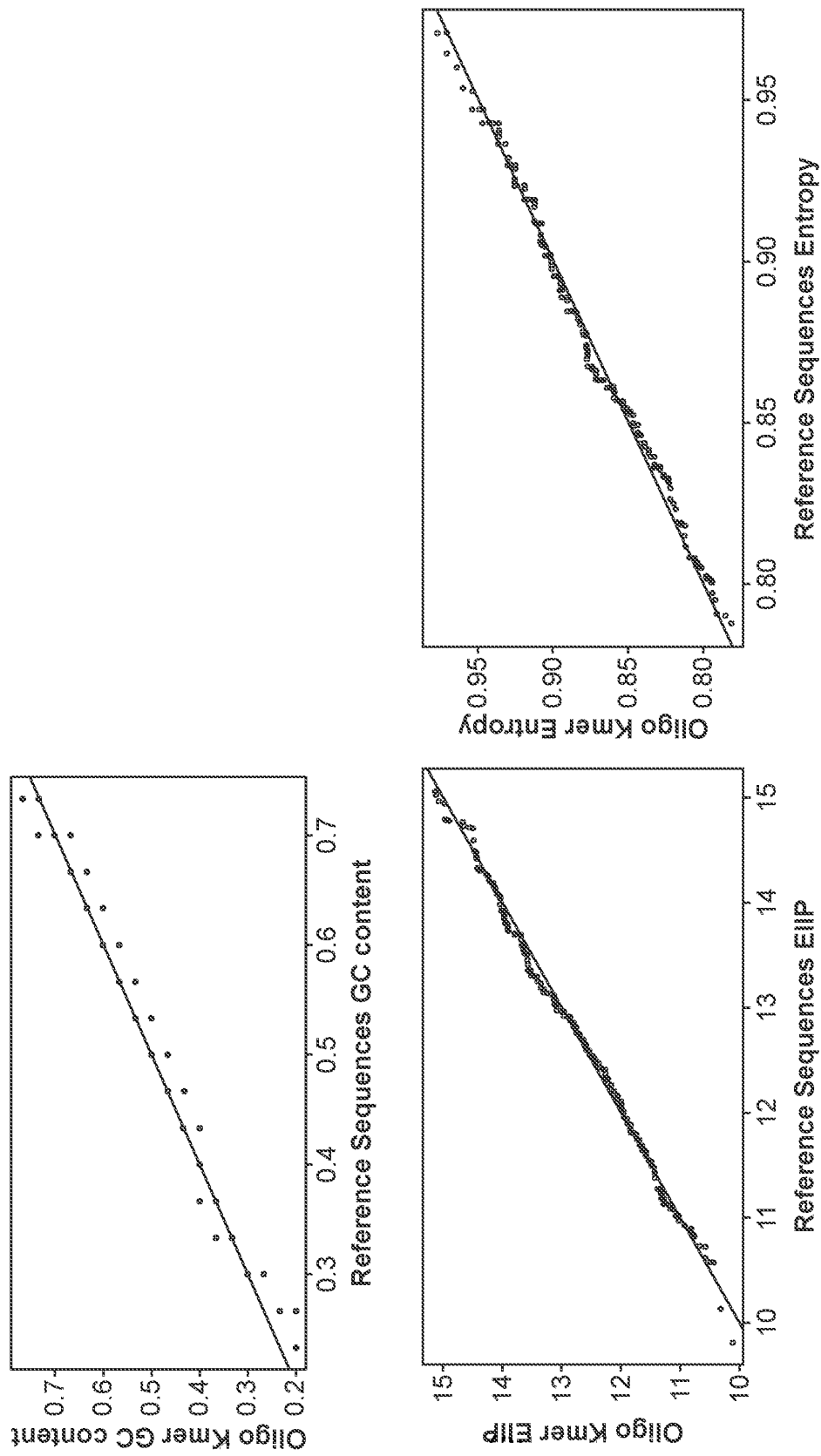
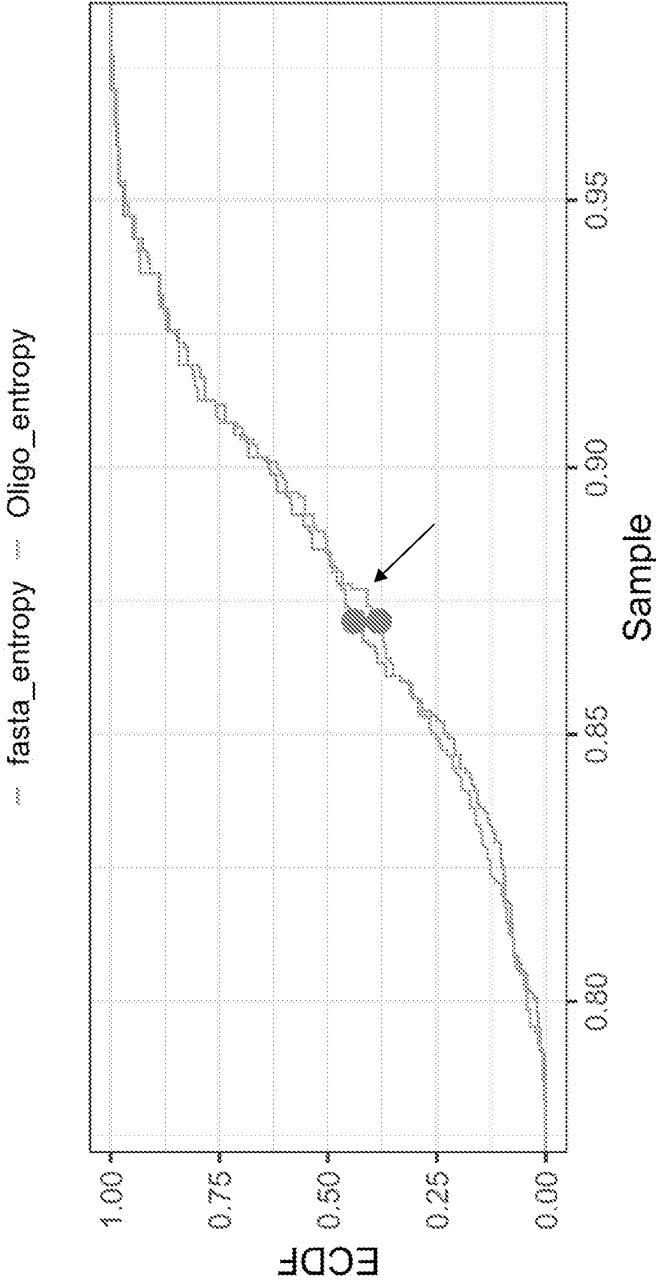


FIG. 23

FIG. 24
K-S Test: Sample 1 / Sample 2



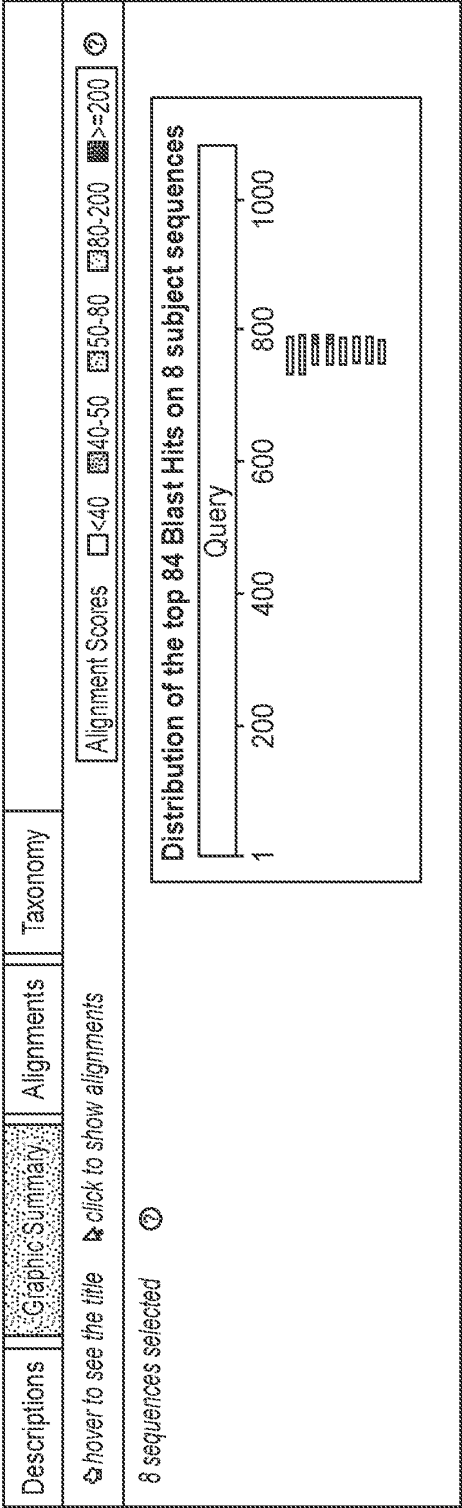


FIG. 25A

Job Title

Nucleotide Sequence

RID

MHKOH18B014 Search expires on 08-07 08:38 am

Download All ▾

Program

🔗

Citation ▾

Database

nr See details ▾

Query ID

ic|Query_226969

Description

None

Molecule type

dna

Query Length

1080

Other reports

🔗

⚠️

No significant similarity found. For reasons why [click here](#)

Filter Results

Organism only top 20 will appear

☐ exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity

to

E value

to

Filter

Reset

FIG. 25B

FIG. 26
Comparison of Cantor Shuffling and Exhaustive Kmer Approaches

Parameters	Cantor Shuffling	Exhaustive search
p value and D statistic(ks test - entropy)	1.0, 0.06 - 0.08	0.7212, 0.0566
p value and D statistic (ks test - eiip)	1.0, 0.01 - 0.044	0.58, 0.0633
p value (mann whitney test - GC content)	0.0039 - 0.49	0.2672
Done on all References Genomes?	NO	YES
Time taken to build		3-4 days
Any further shuffling required?	YES - to mask regions	NO
features calculated on all References Genomes?	NO	YES

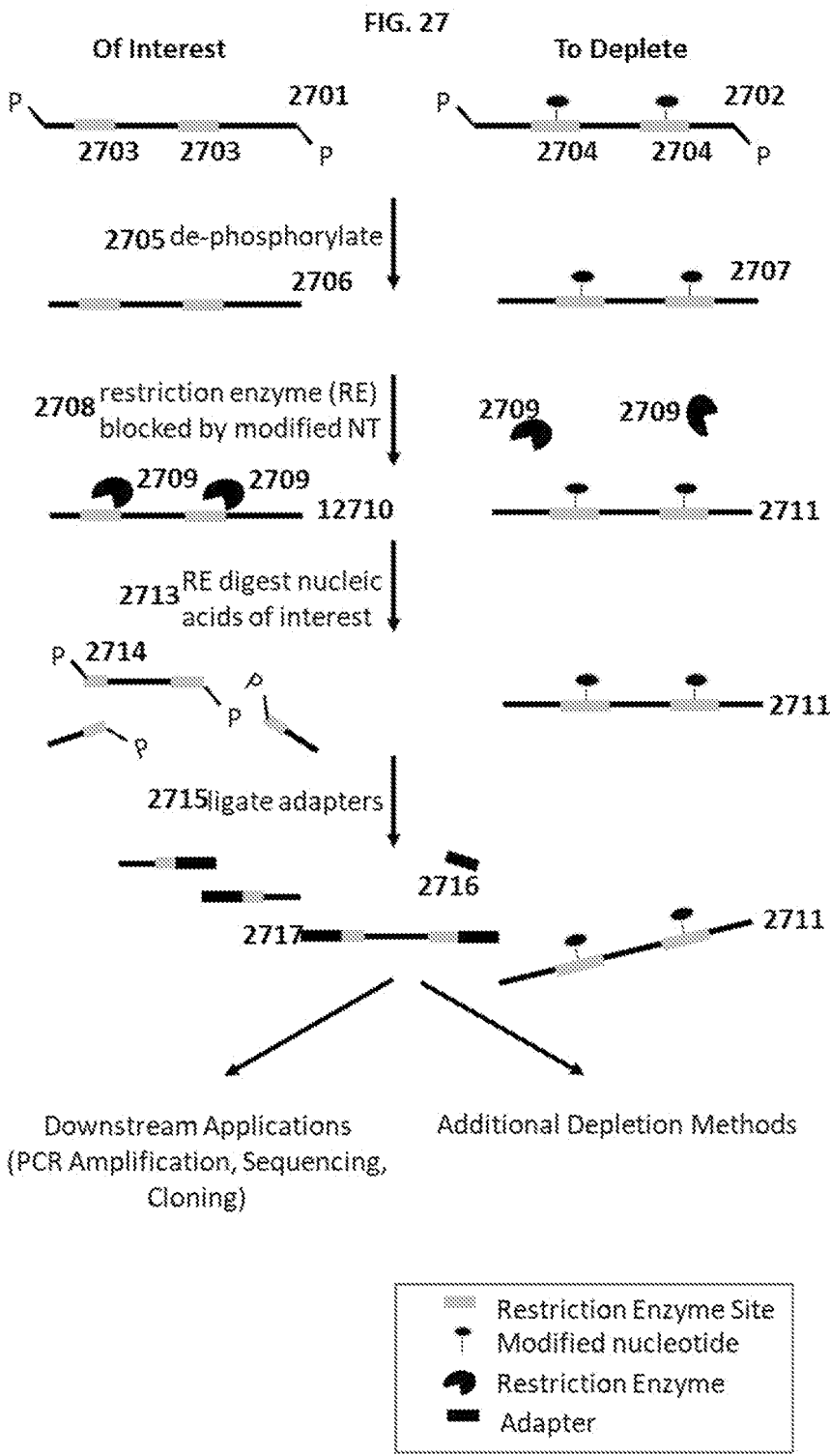


FIG. 28

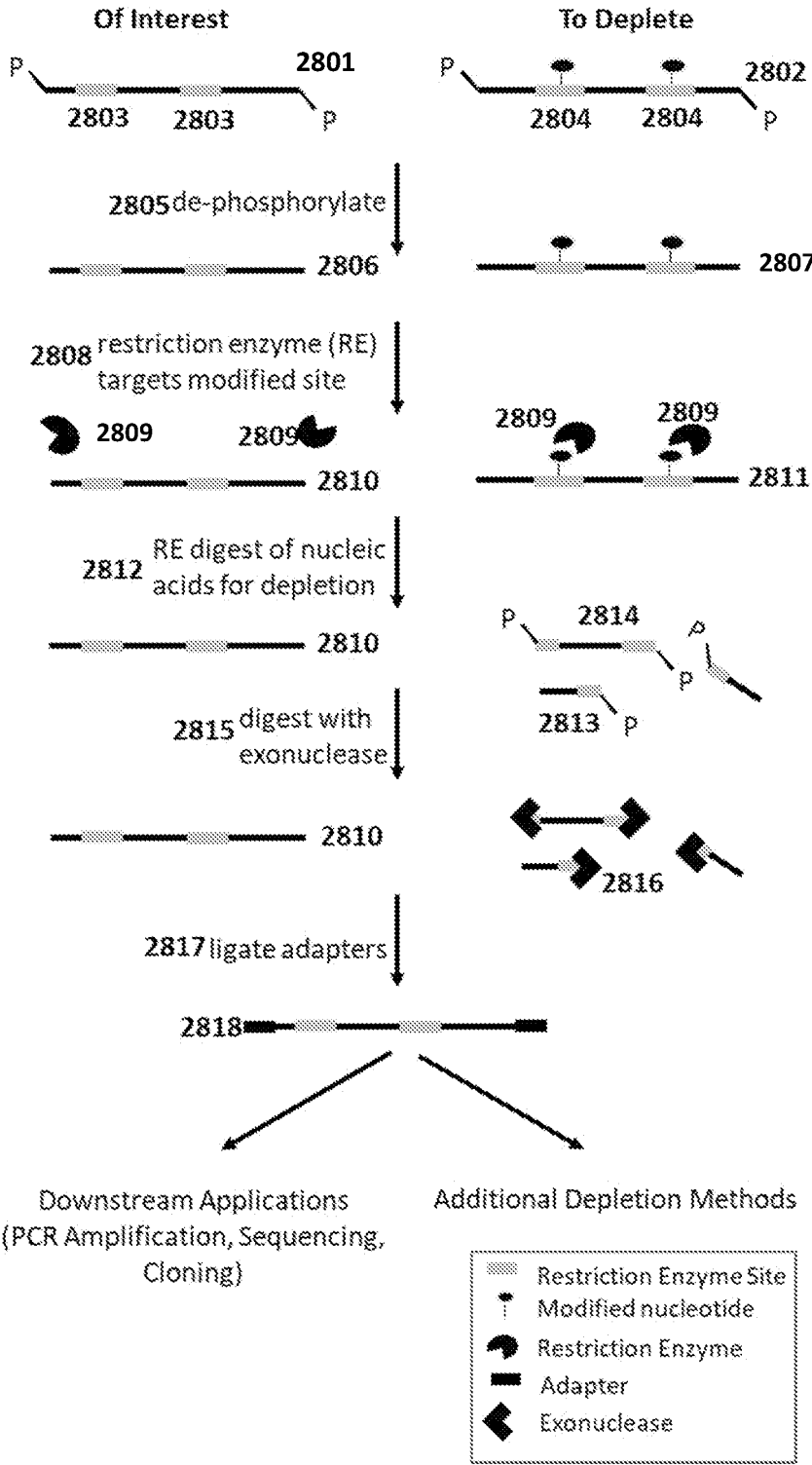


FIG. 29

