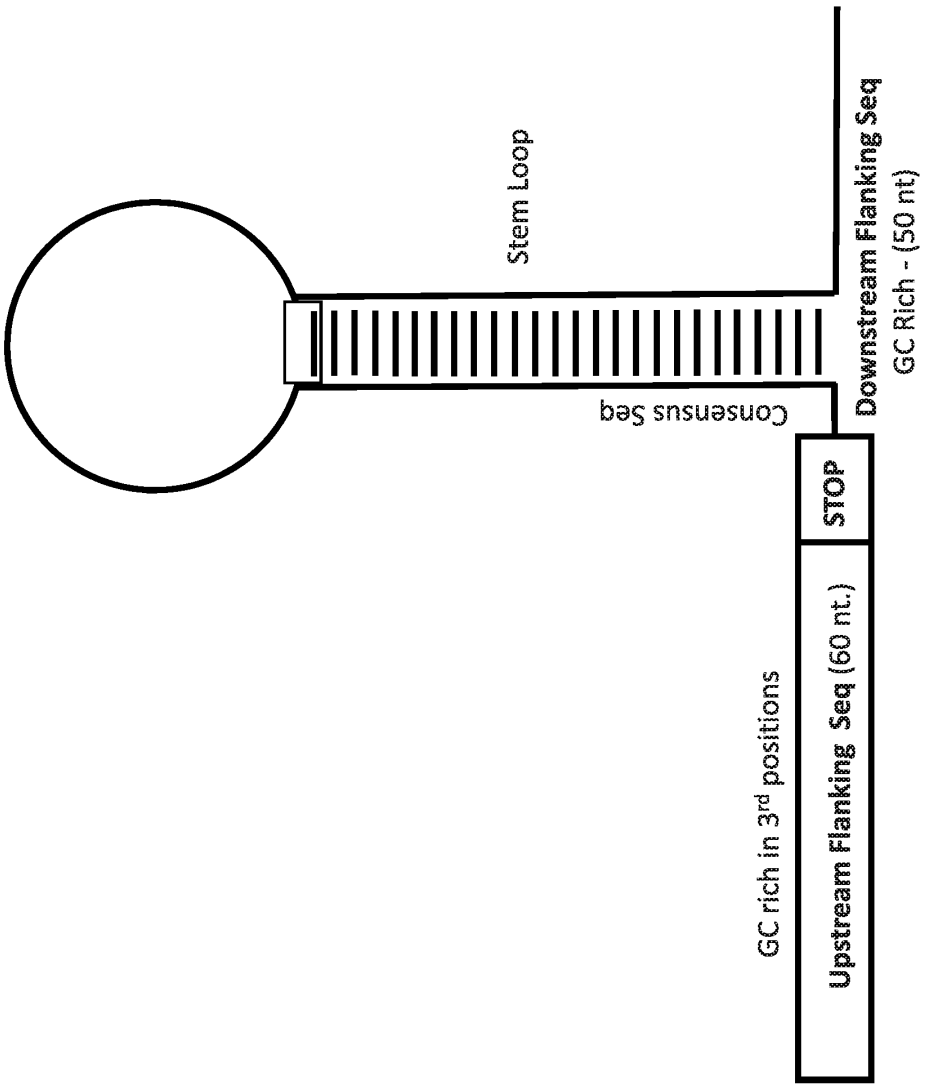
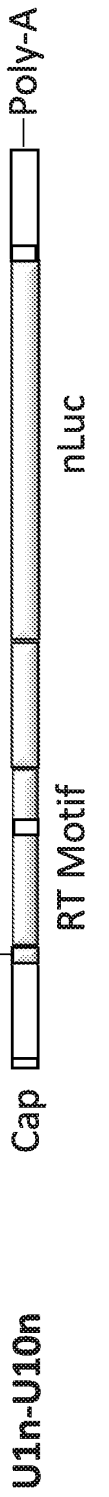

Stem Loop
Downstream Flanking Seq
GC Rich - (50 nt)
Consensus Seq
STOP
Upstream Flanking Seq (60 nt.)
GC rich in 3rd positions

FIG. 1



Construct Map

RT Motif: Onco-selective Readthrough (RT) motif
2A: Self-cleaving peptide (P2A and T2A with linker in between)
nLuc: nano-luciferase without ATG (luminescence marker)

FIG. 2

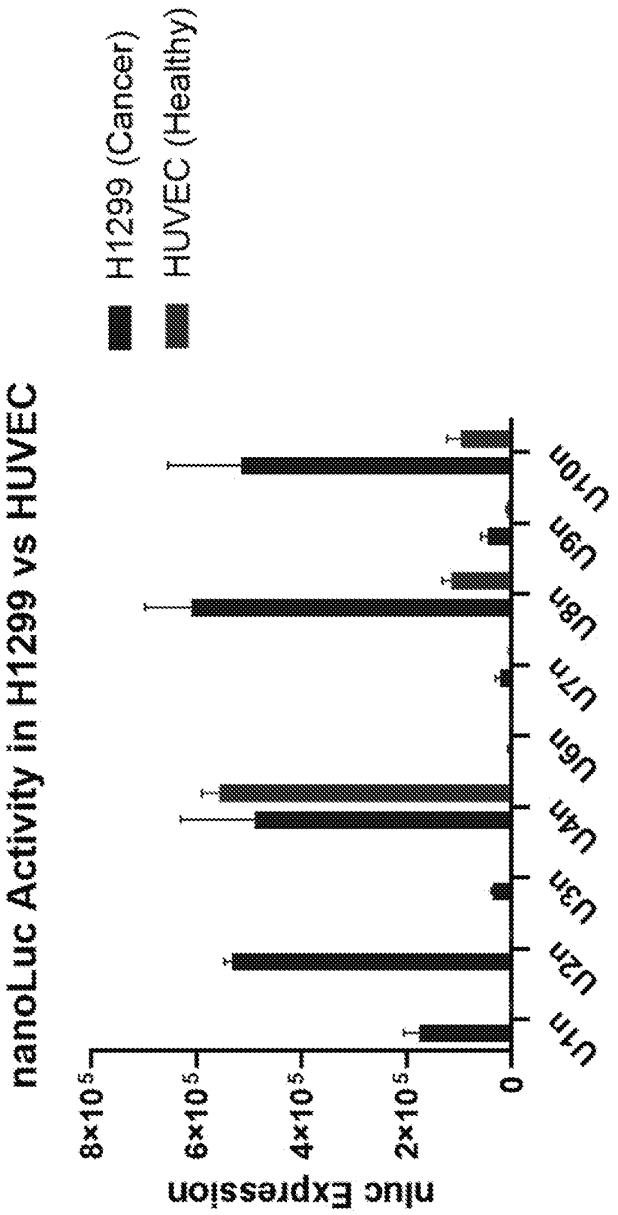

nanoLuc Activity in H1299 vs HUVEC
H1299 (Cancer)
HUVEC (Healthy)
nluc Expression
8×10^5
6×10^5
4×10^5
2×10^5
0
U1n
U2n
U3n
U4n
U6n
U7n
U8n
U9n
U10n
Construct

FIG. 3

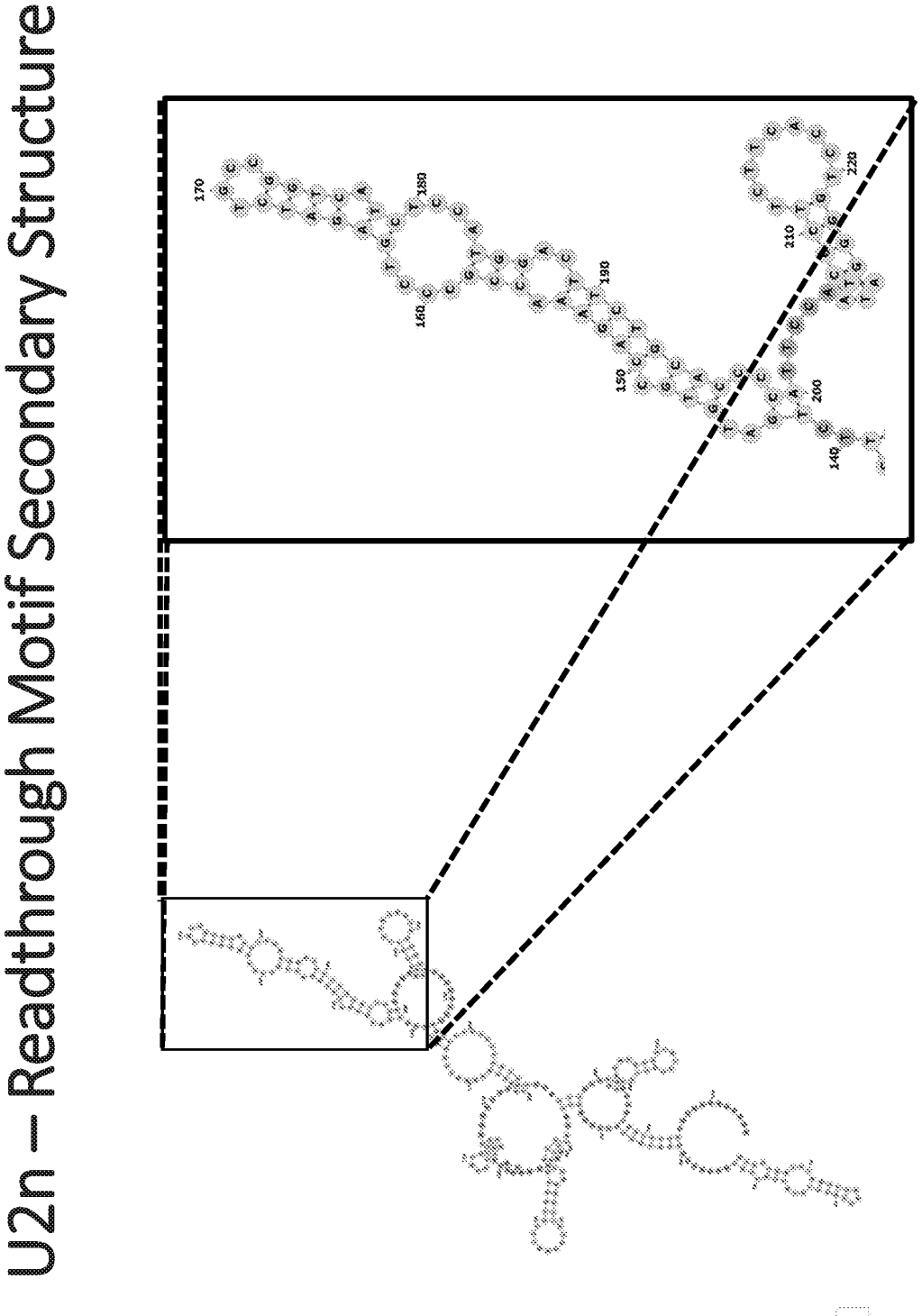
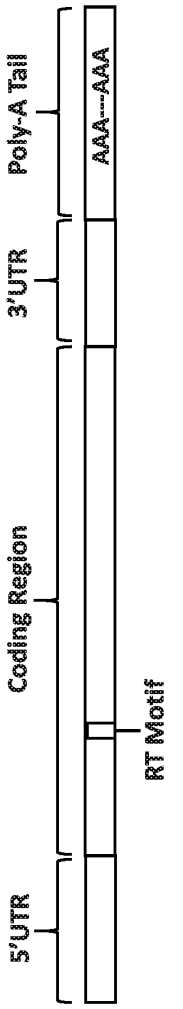


FIG. 4



Onco-333

RT Motif: Onco-selective Readthrough (RT) motif

5'UTR: 5' Untranslated Region

3'UTR: 3' Untranslated Region

FIG. 5

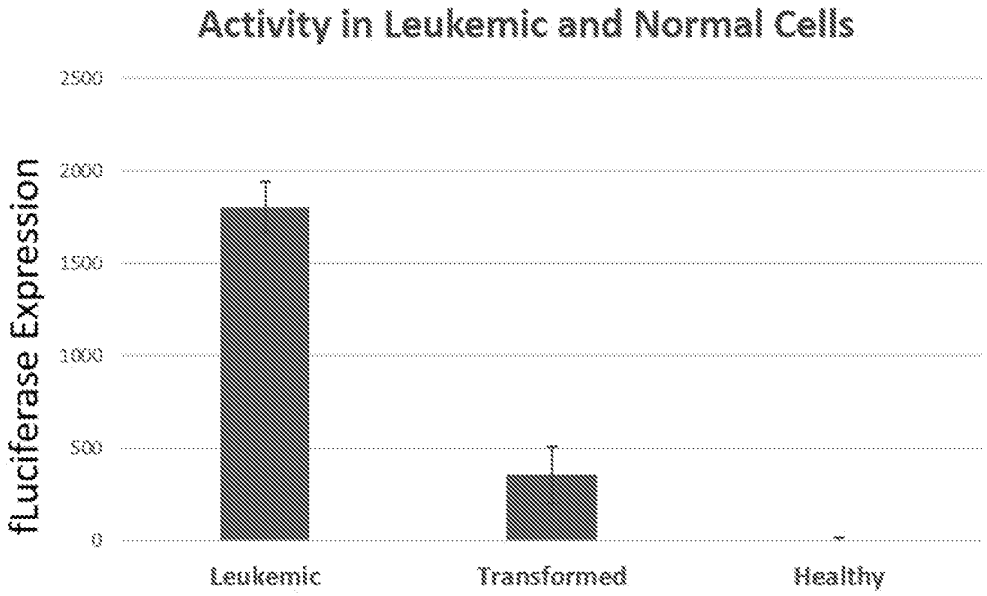

Activity in Leukemic and Normal Cells
fLuciferase Expression
2500
2000
1500
1000
500
0
Leukemic
Transformed
Healthy

FIG. 6

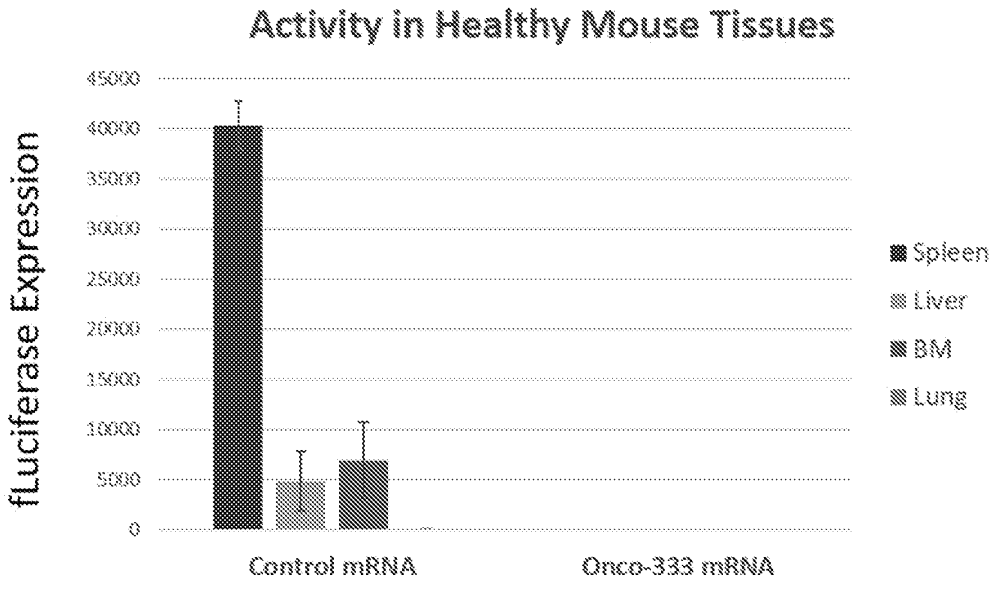

Activity in Healthy Mouse Tissues
fLuciferase Expression
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Control mRNA
Onco-333 mRNA
Spleen
Liver
BM
Lung

FIG. 7

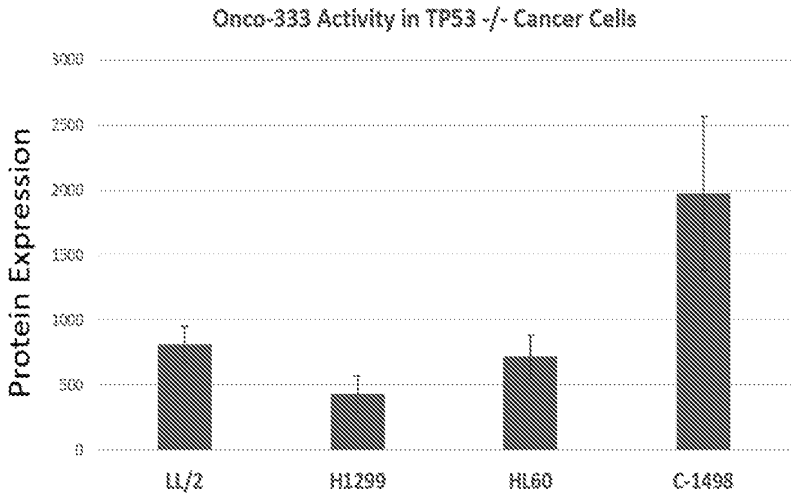

Onco-333 Activity in TP53 -/- Cancer Cells
Protein Expression
3000
2500
2000
1500
1000
500
0
LL/2
H1299
HL60
C-1498

FIG. 8

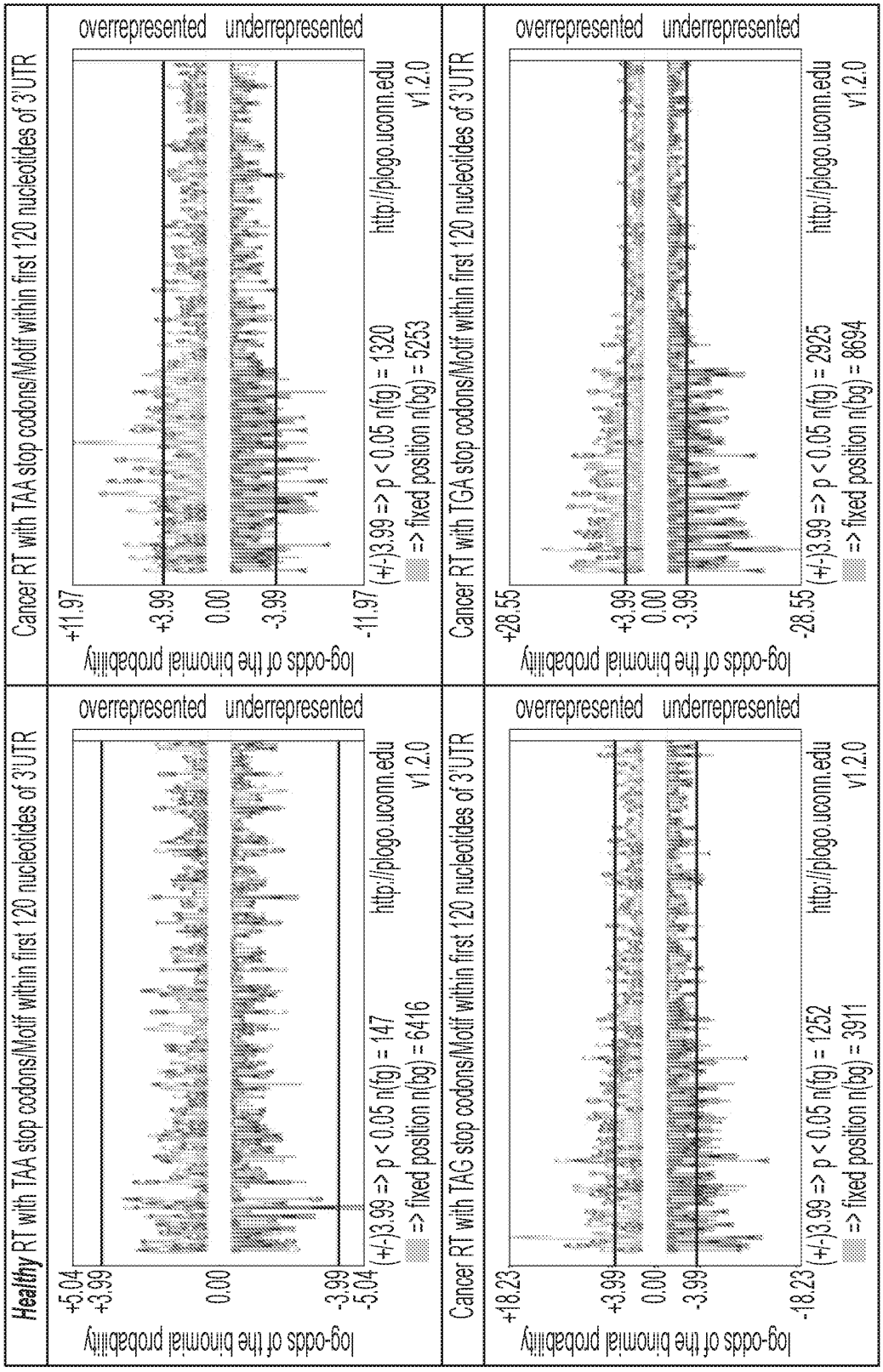

Healthy RT with TAA stop codons/Motif within first 120 nucleotides of 3'UTR
Cancer RT with TAA stop codons/Motif within first 120 nucleotides of 3'UTR
Cancer RT with TAG stop codons/Motif within first 120 nucleotides of 3'UTR
Cancer RT with TGA stop codons/Motif within first 120 nucleotides of 3'UTR
overrepresented
underrepresented
log-odds of the binomial probability
+5.04
+3.99
0.00
-3.99
-5.04
+11.97
-11.97
+18.23
-18.23
+28.55
-28.55
(+/-)3.99 => p < 0.05 n(fg) = 147
=> fixed position n(bg) = 6416
(+/-)3.99 => p < 0.05 n(fg) = 1320
=> fixed position n(bg) = 5253
(+/-)3.99 => p < 0.05 n(fg) = 1252
=> fixed position n(bg) = 3911
(+/-)3.99 => p < 0.05 n(fg) = 2925
=> fixed position n(bg) = 8694
http://plogo.uconn.edu
v1.2.0

FIG. 9

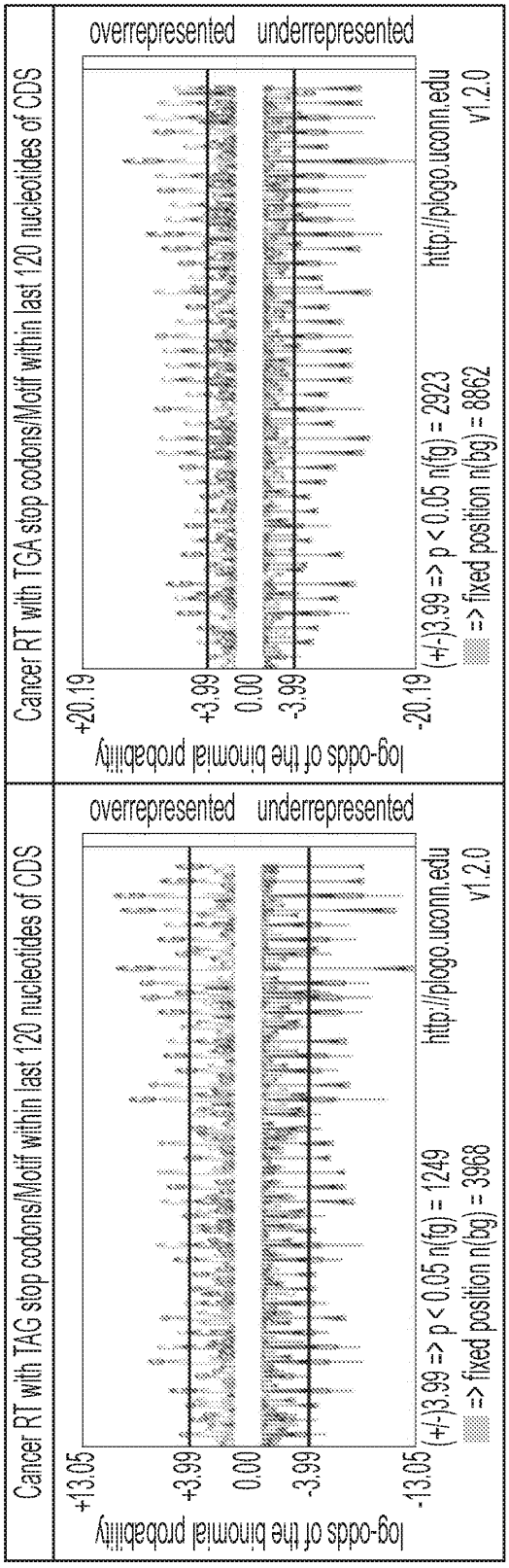

Cancer RT with TAG stop codons/Motif within last 120 nucleotides of CDS
overrepresented
underrepresented
log-odds of the binomial probability
+13.05
+3.99
0.00
-3.99
-13.05
(+/-)3.99 => p < 0.05 n(fg) = 1249
=> fixed position n(bg) = 3968
http://plogo.uconn.edu
v1.2.0
Cancer RT with TGA stop codons/Motif within last 120 nucleotides of CDS
overrepresented
underrepresented
log-odds of the binomial probability
+20.19
+3.99
0.00
-3.99
-20.19
(+/-)3.99 => p < 0.05 n(fg) = 2923
=> fixed position n(bg) = 8862
http://plogo.uconn.edu
v1.2.0

FIG. 10

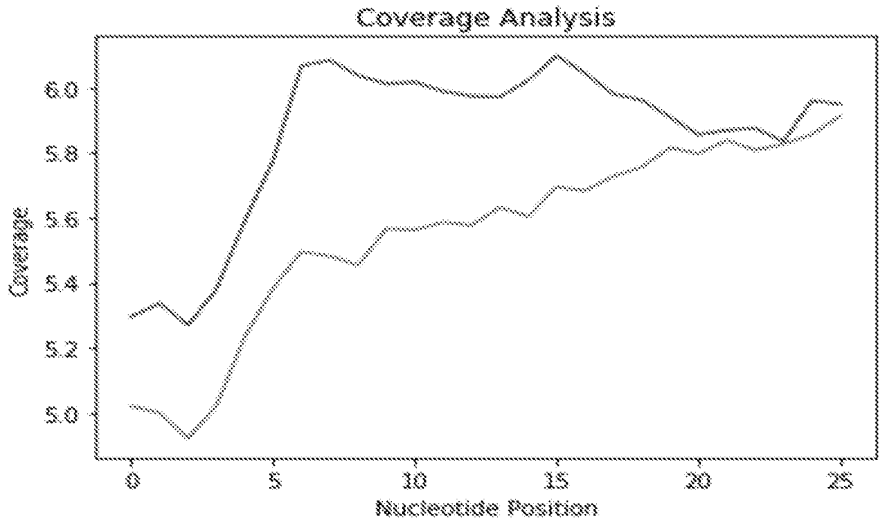

Coverage Analysis
Coverage
6.0
5.8
5.6
5.4
5.2
5.0
0
5
10
15
20
25
Nucleotide Position

FIG. 11

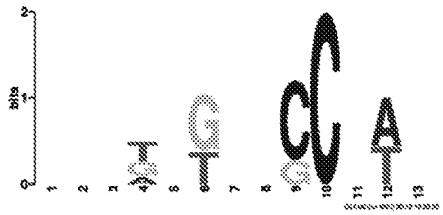
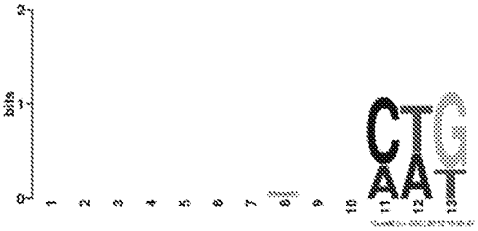
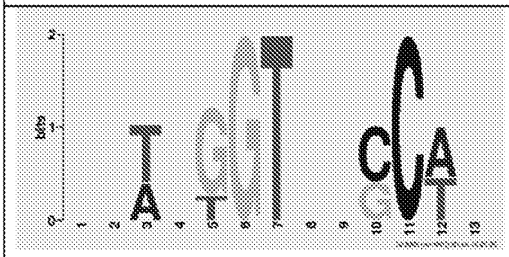
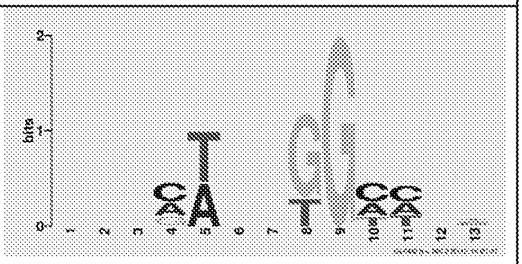
Healthy RT with TAA stop codons/Motif within first 13 nucleotides of 3'UTR	Cancer RT with TAA stop codons/Motif within first 13 nucleotides of 3'UTR
	
Regular Expression for Motif: .?.?.?[tg].?[tg].?.?[cg]c?.?[at].? NNNDNKNNSCNWN	*Regular Expression for Motif:* [acg].?.?.?.?.?.?[ac].?.?[ac][at][tg] VNNNNNNMNNMWK
	

FIG. 12

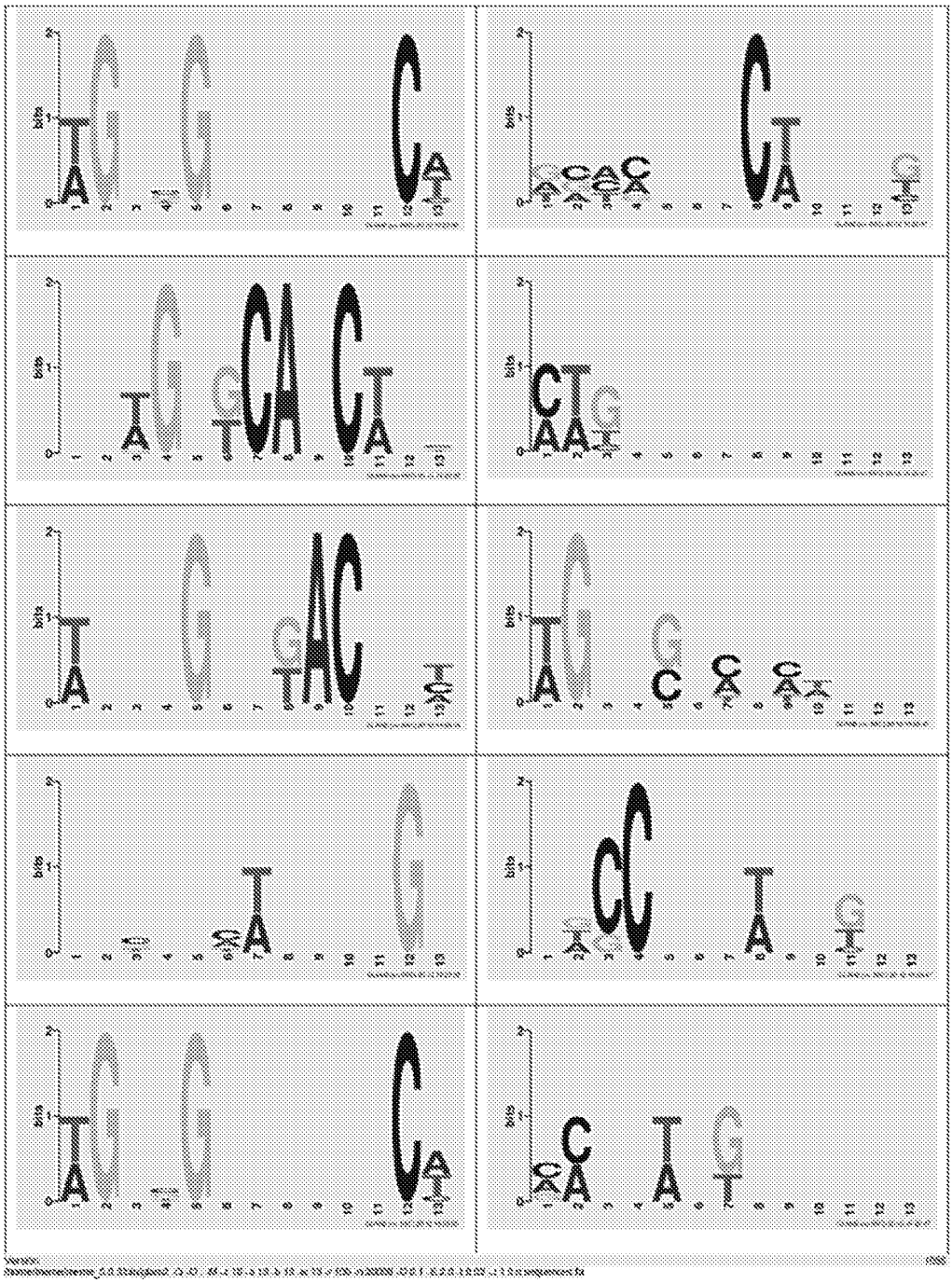


FIG. 13

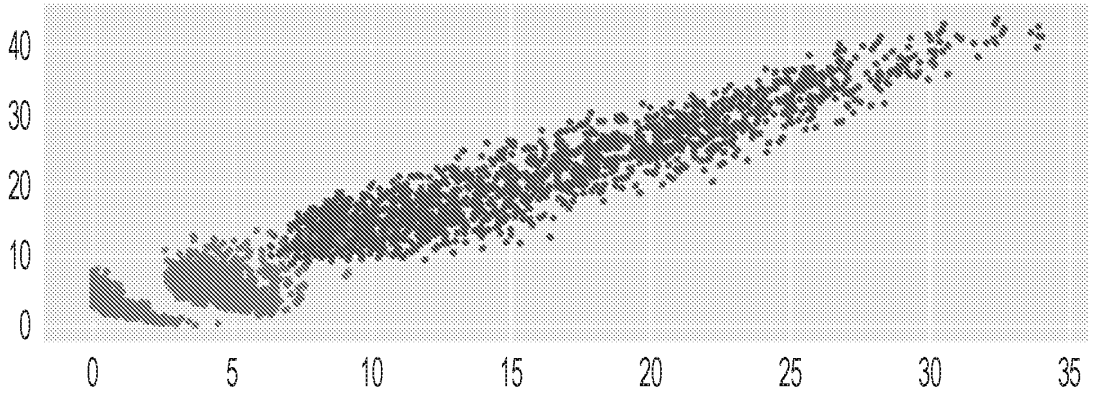


FIG. 14

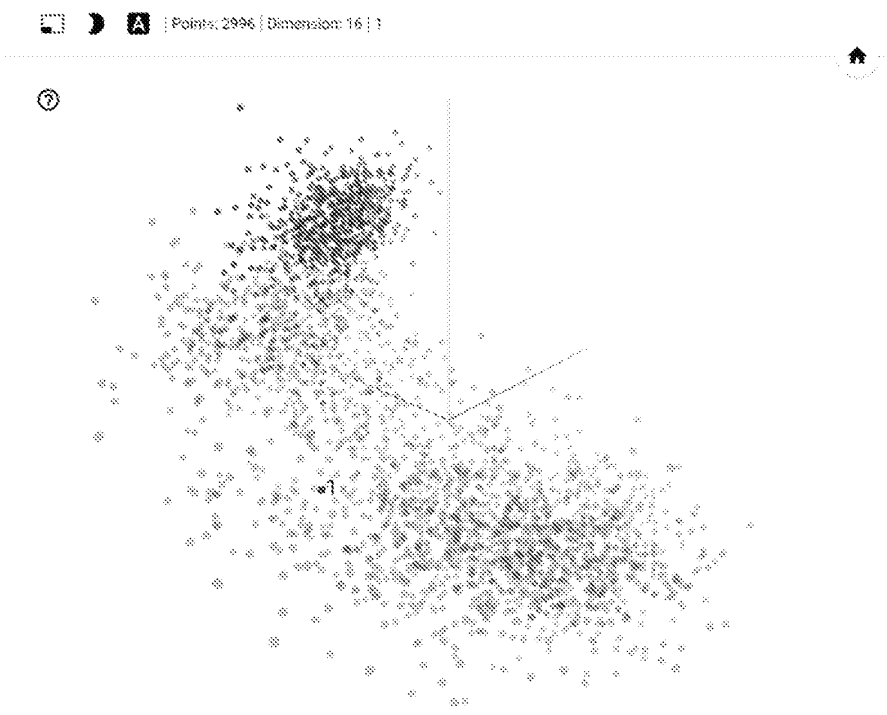


FIG. 15

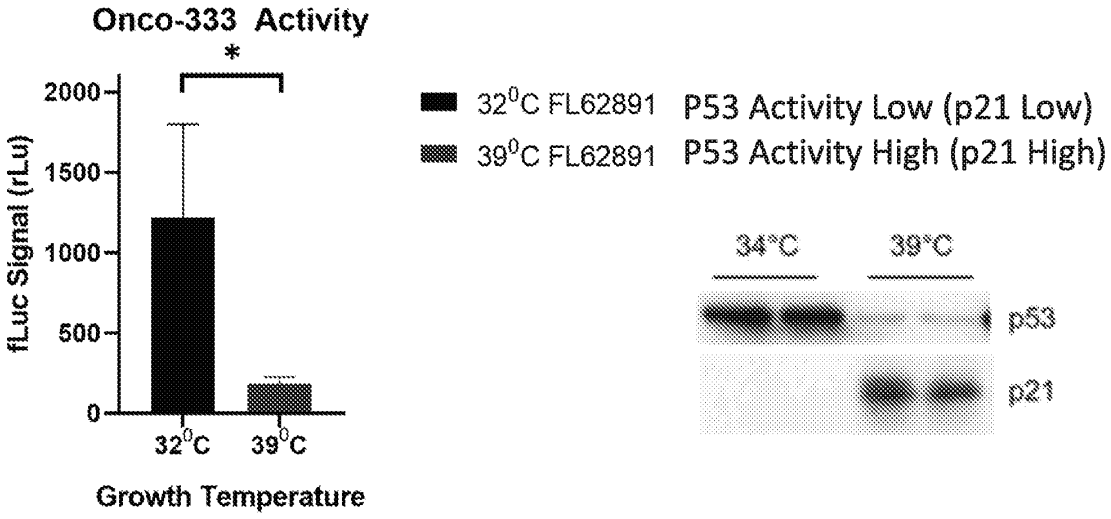

Onco-333 Activity
*
2000
1500
1000
500
0
fLuc Signal (rLu)
32°C
39°C
Growth Temperature
32°C FL62891 P53 Activity Low (p21 Low)
39°C FL62891 P53 Activity High (p21 High)
34°C
39°C
p53
p21

FIG. 16

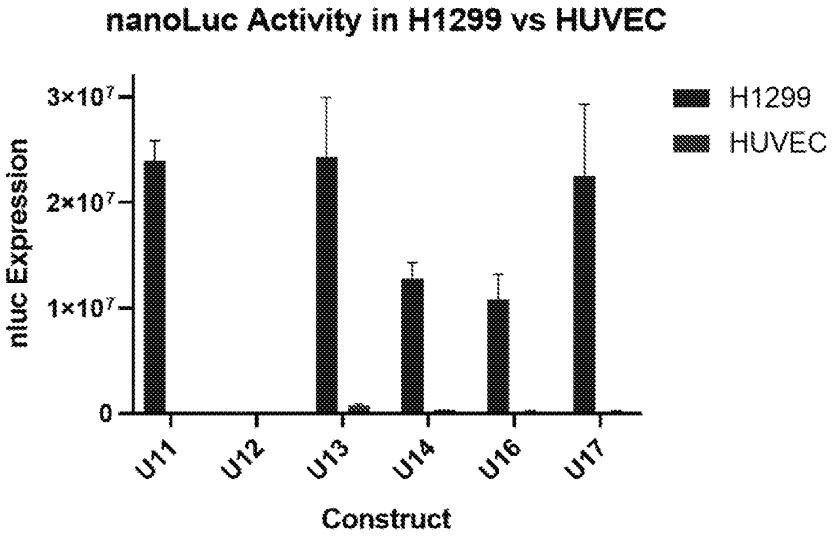


FIG. 17A

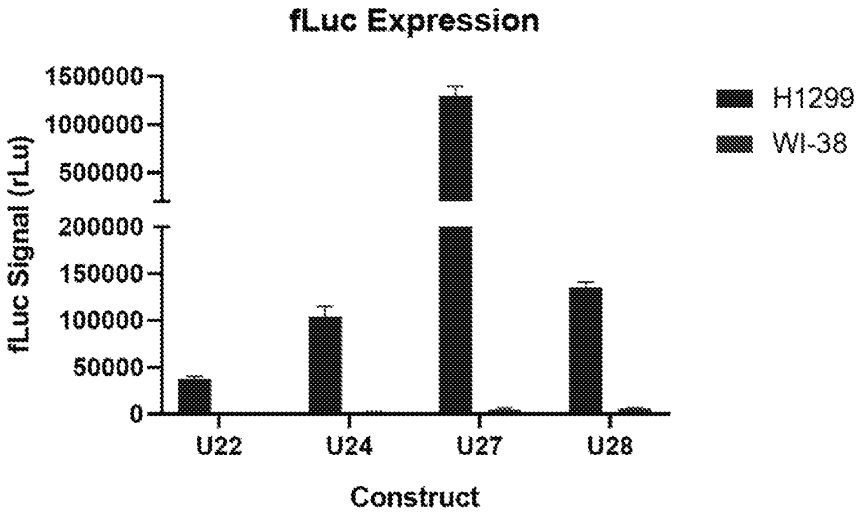


FIG. 17B