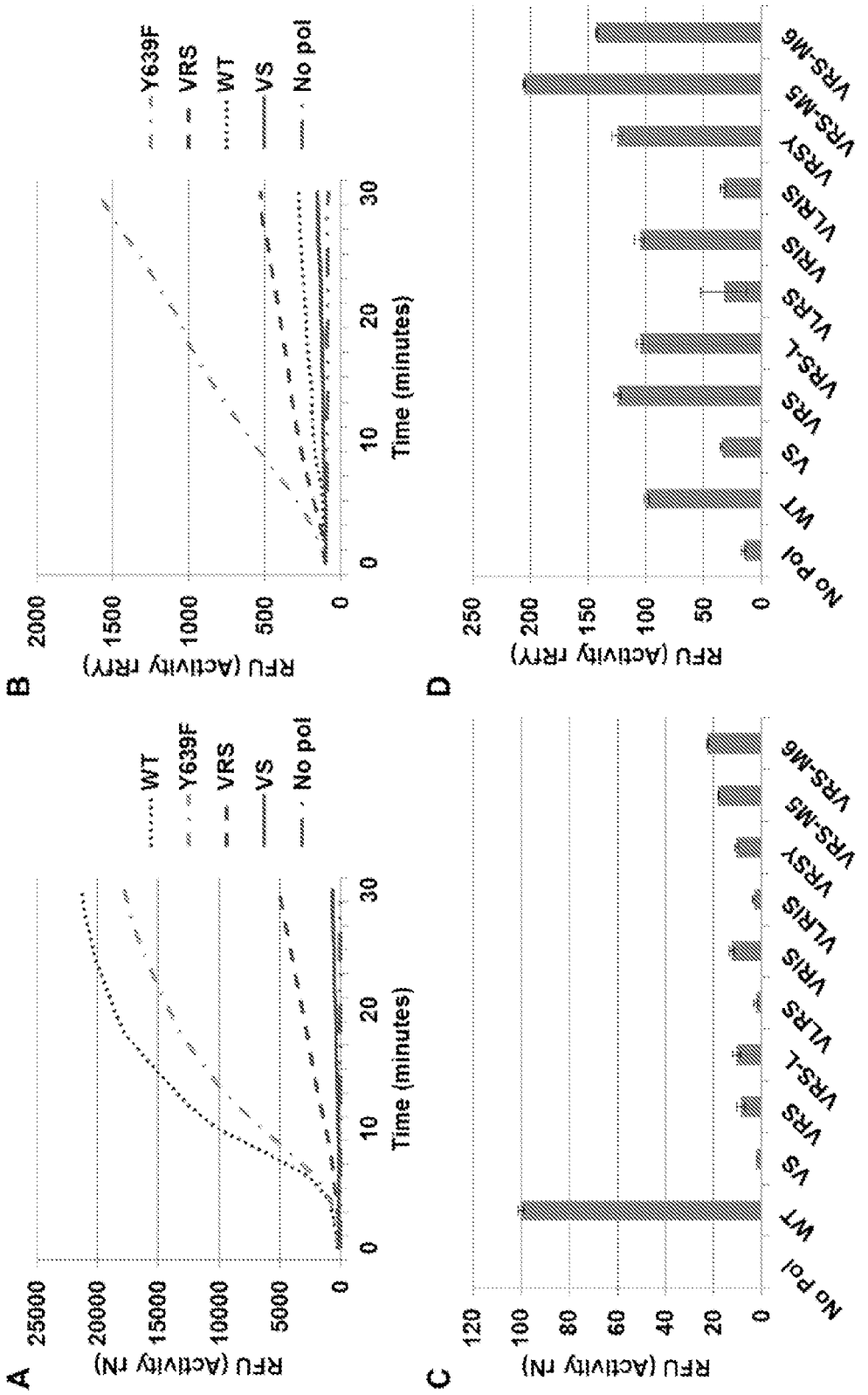
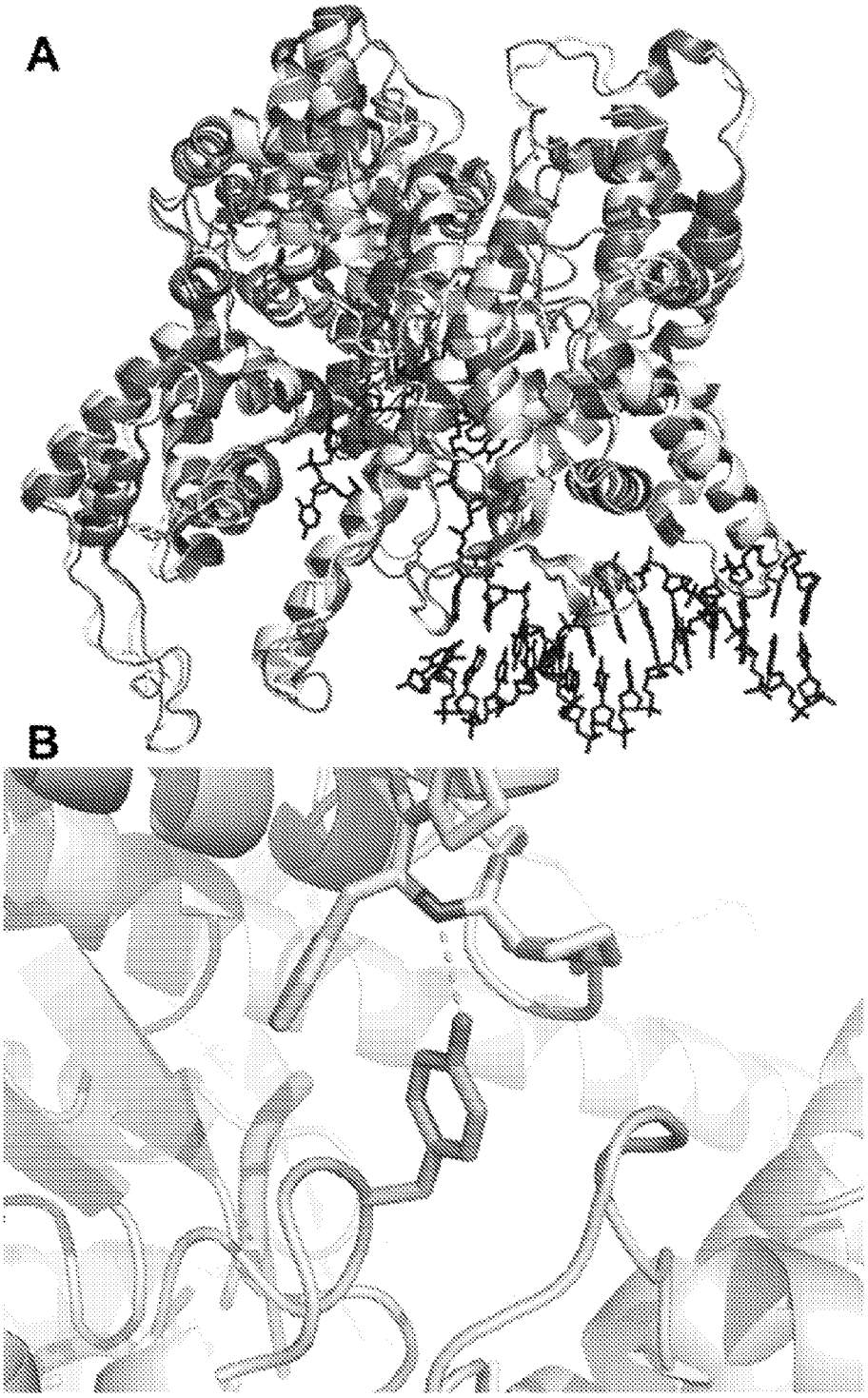
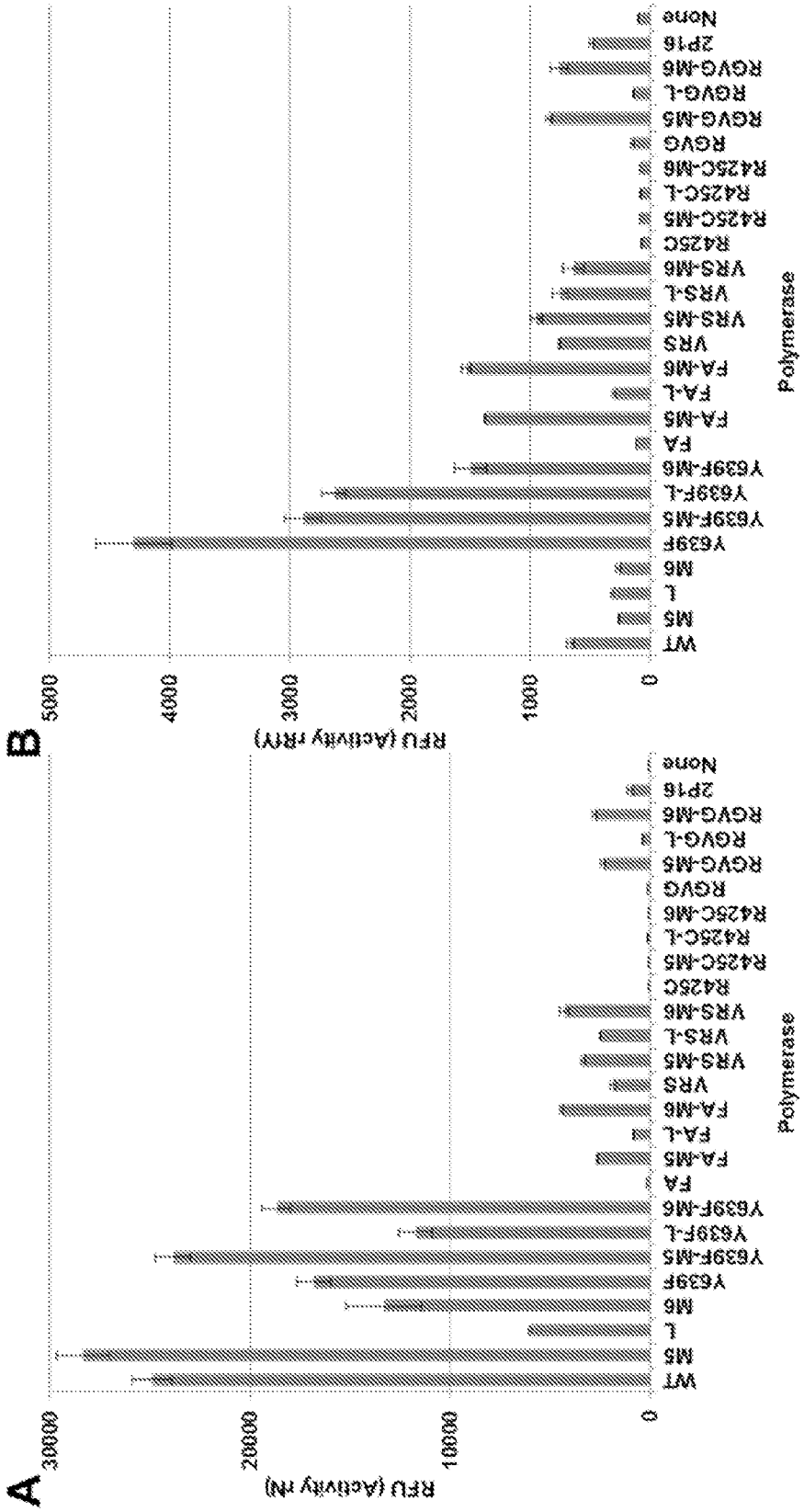

A
RFU (Activity rN)
25000
20000
15000
10000
5000
0
0
10
20
30
Time (minutes)
WT
Y639F
VRS
VS
No pol
B
RFU (Activity rRTY)
2000
1500
1000
500
0
C
RFU (Activity rN)
120
100
80
60
40
20
0
D
RFU (Activity rRTY)
250
200
150
100
50
0
No Pol
WT
VS
VRS
VRS-L
VLRS
VRIS
VLRIS
VRSY
VRS-M5
VRS-M6

FIG. 1A – 1D



FIGS. 2A – 2B


A
RFU (Activity nM)
30000
20000
10000
0
WT
M5
L
M6
Y639F
Y639F-M5
Y639F-L
Y639F-M6
FA
FA-M5
FA-L
FA-M6
VRS
VRS-M5
VRS-L
VRS-M6
R425C
R425C-M5
R425C-L
R425C-M6
RGVG
RGVG-M5
RGVG-L
RGVG-M6
2P16
None
Polymerase
B
RFU (Activity nRY)
5000
4000
3000
2000
1000
0
WT
M5
L
M6
Y639F
Y639F-M5
Y639F-L
Y639F-M6
FA
FA-M5
FA-L
FA-M6
VRS
VRS-M5
VRS-L
VRS-M6
R425C
R425C-M5
R425C-L
R425C-M6
RGVG
RGVG-M5
RGVG-L
RGVG-M6
2P16
None
Polymerase

FIGS. 3A – 3B

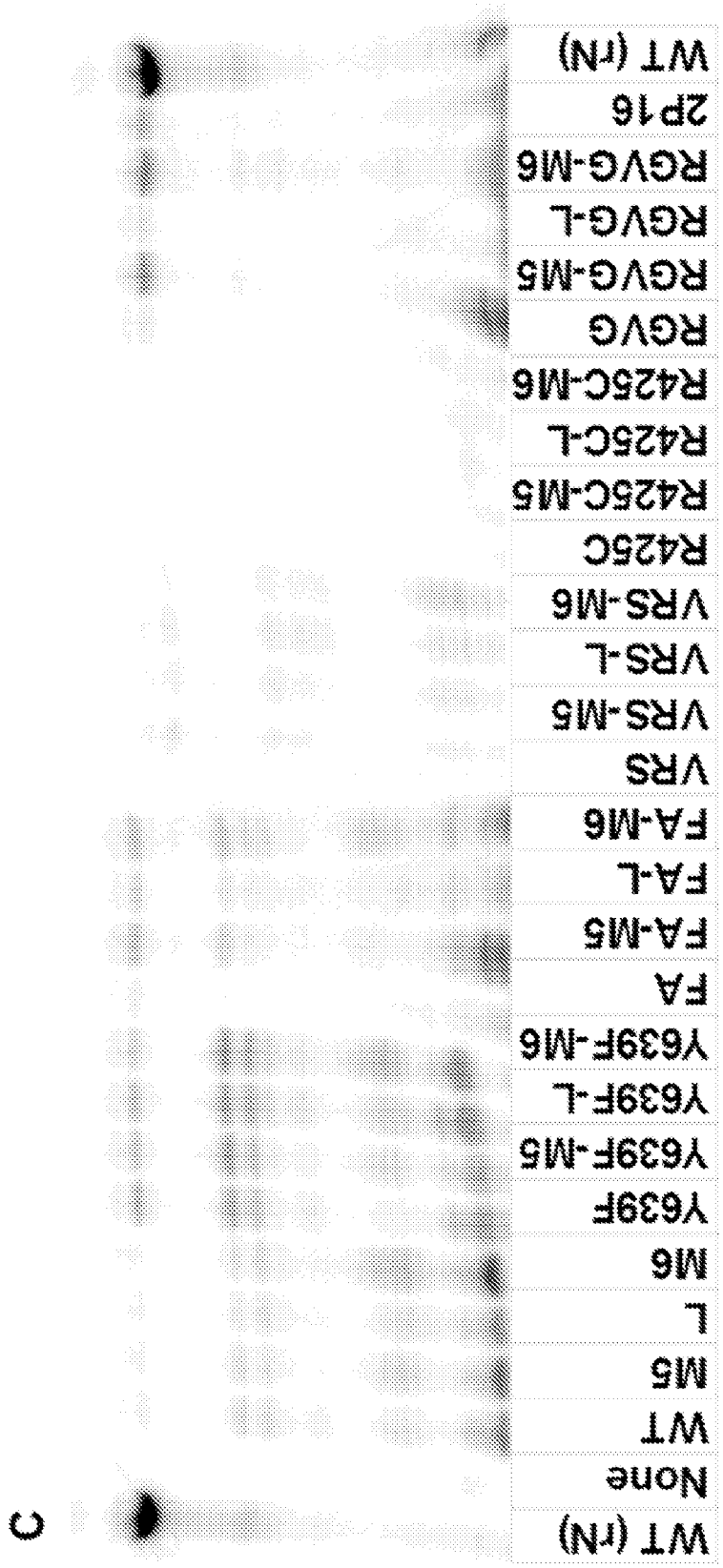
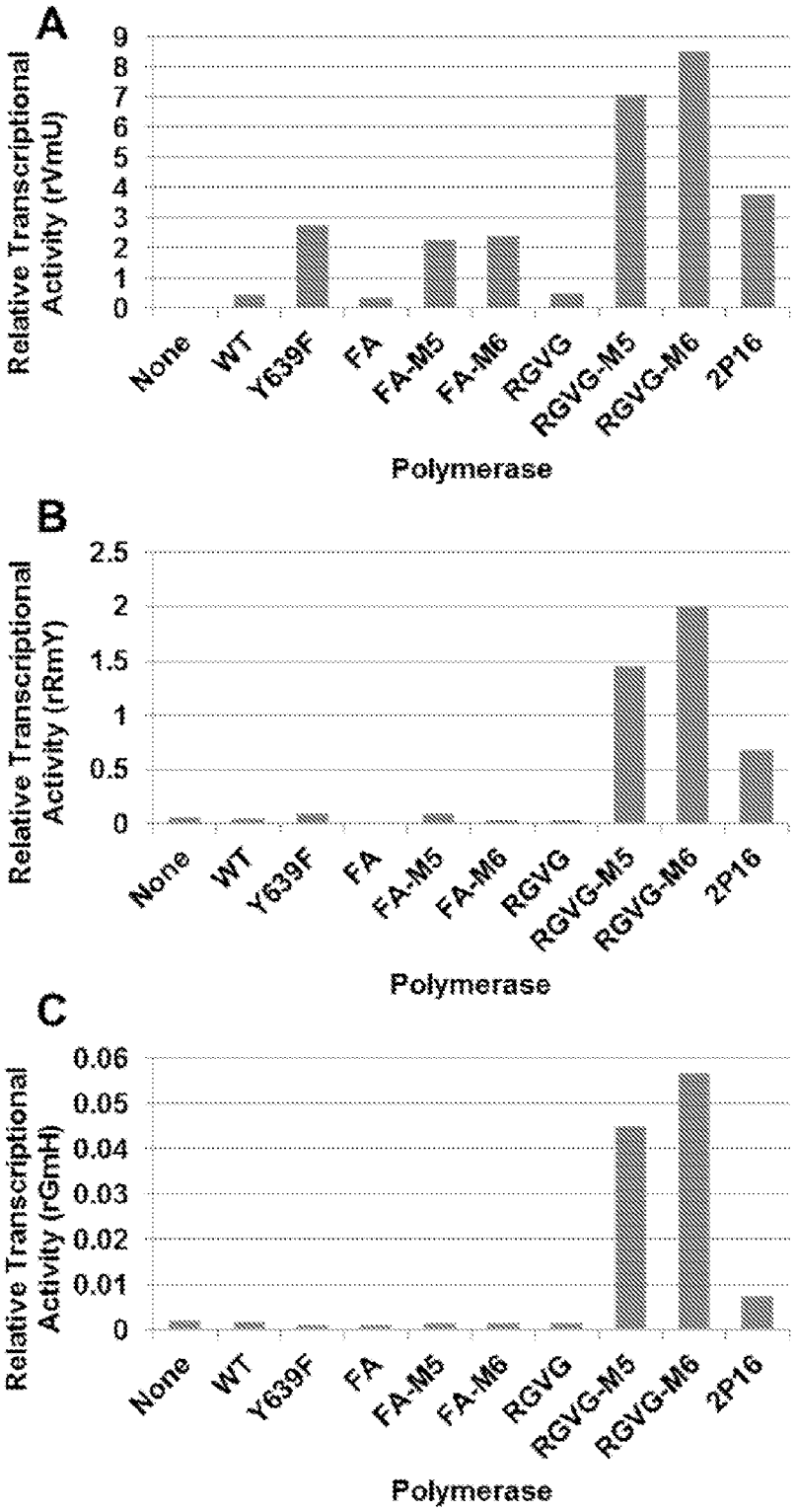

C
WT (rN)
None
WT
M5
L
M6
Y639F
Y639F-M5
Y639F-L
Y639F-M6
FA
FA-M5
FA-L
FA-M6
VRS
VRS-M5
VRS-L
VRS-M6
R425C
R425C-M5
R425C-L
R425C-M6
RGVG
RGVG-M5
RGVG-L
RGVG-M6
2P16
WT (rN)

FIG. 3C


A
Relative Transcriptional Activity (rVmU)
9
8
7
6
5
4
3
2
1
0
None
WT
Y639F
FA
FA-M5
FA-M6
RGVG
RGVG-M5
RGVG-M6
2P16
Polymerase
B
Relative Transcriptional Activity (rRmY)
2.5
2
1.5
1
0.5
0
None
WT
Y639F
FA
FA-M5
FA-M6
RGVG
RGVG-M5
RGVG-M6
2P16
Polymerase
C
Relative Transcriptional Activity (rGmH)
0.06
0.05
0.04
0.03
0.02
0.01
0
None
WT
Y639F
FA
FA-M5
FA-M6
RGVG
RGVG-M5
RGVG-M6
2P16
Polymerase

FIGS. 4A – 4C

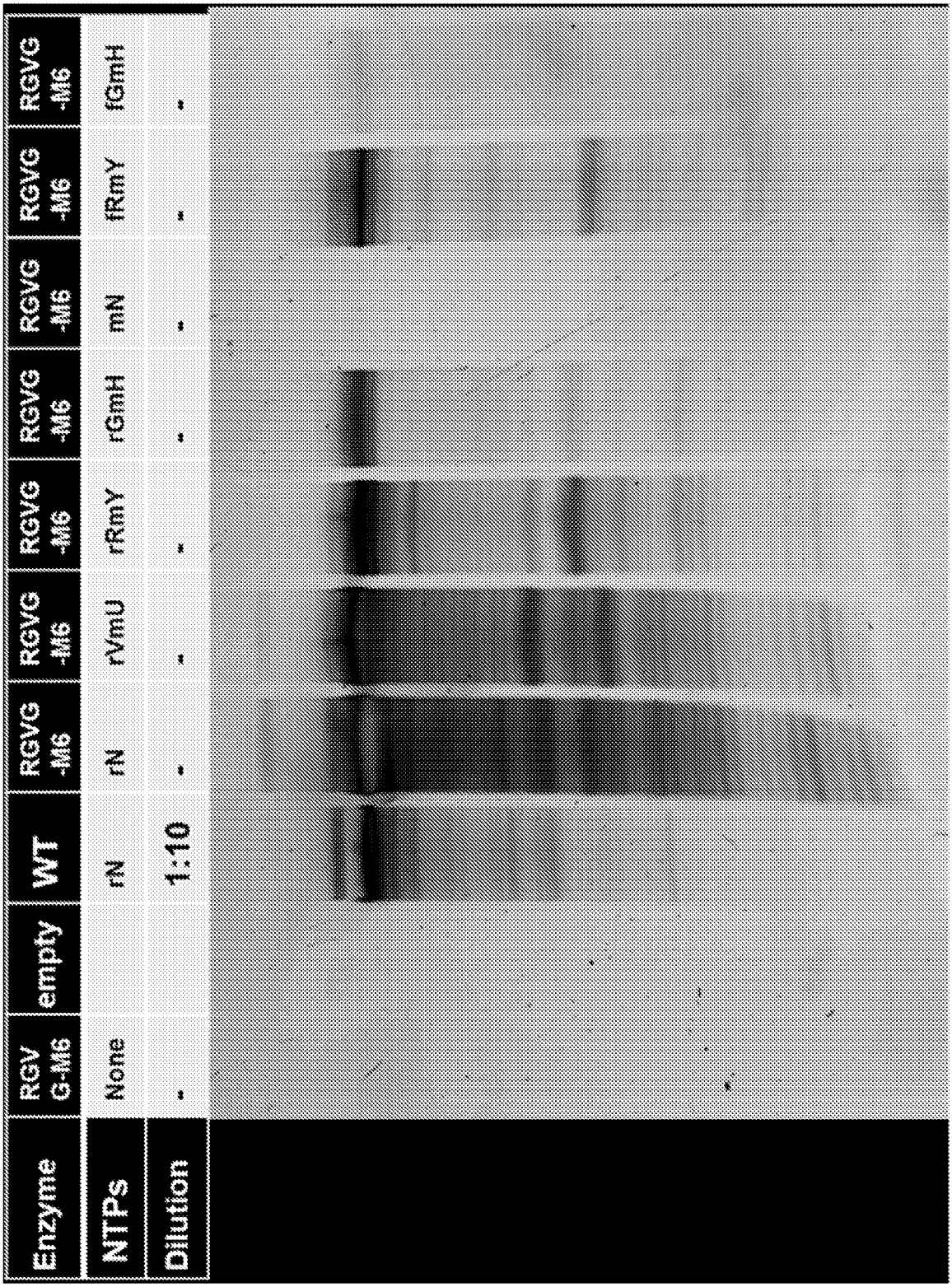

Enzyme
RGV G-M6
empty
WT
RGVG -M6
RGVG -M6
RGVG -M6
RGVG -M6
RGVG -M6
RGVG -M6
NTPs
None
rN
rN
rVmU
rRmY
rGmH
mN
fRmY
fGmH
Dilution
"
1:10
"
"
"
"
"
"

FIG. 5

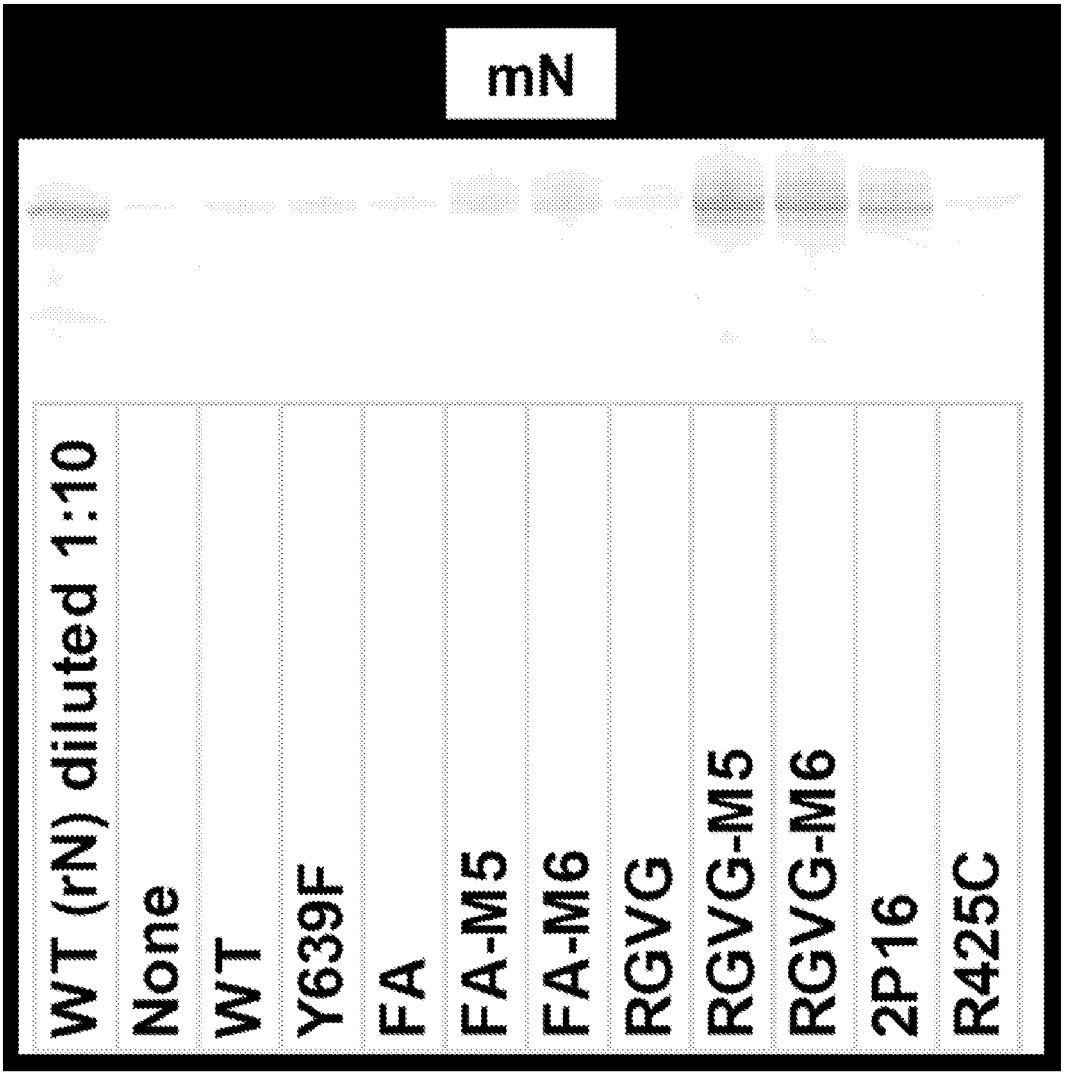

mN
WT (rN) diluted 1:10
None
WT
Y639F
FA
FA-M5
FA-M6
RGVG
RGVG-M5
RGVG-M6
2P16
R425C

FIG. 6

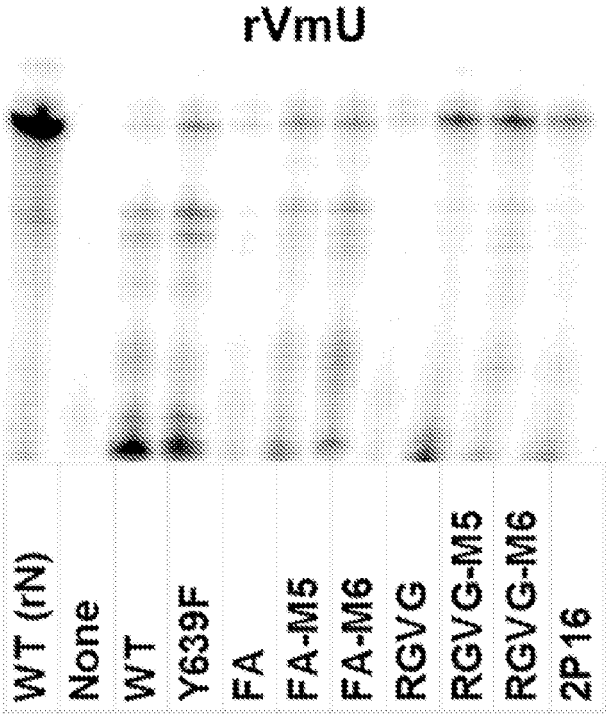

rVmU
WT (rN)
None
WT
Y639F
FA
FA-M5
FA-M6
RGVG
RGVG-M5
RGVG-M6
2P16

FIG. 7

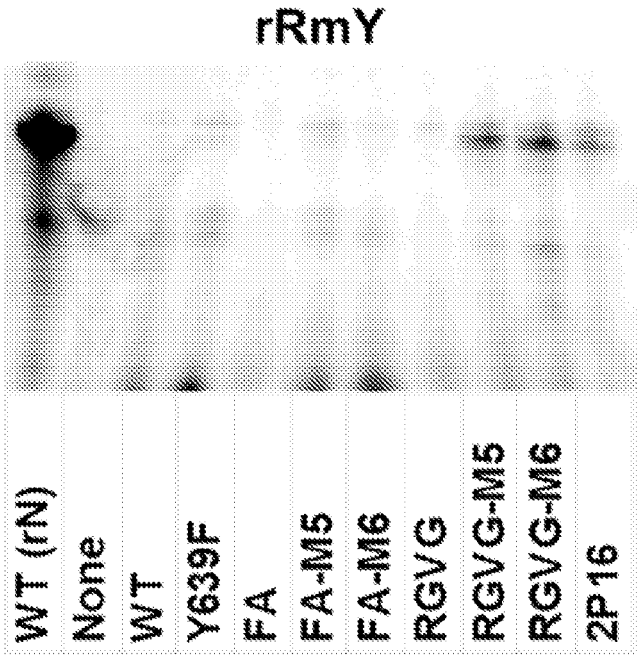

rRmY
WT (rN)
None
WT
Y639F
FA
FA-M5
FA-M6
RGVG
RGVG-M5
RGVG-M6
2P16

FIG. 8

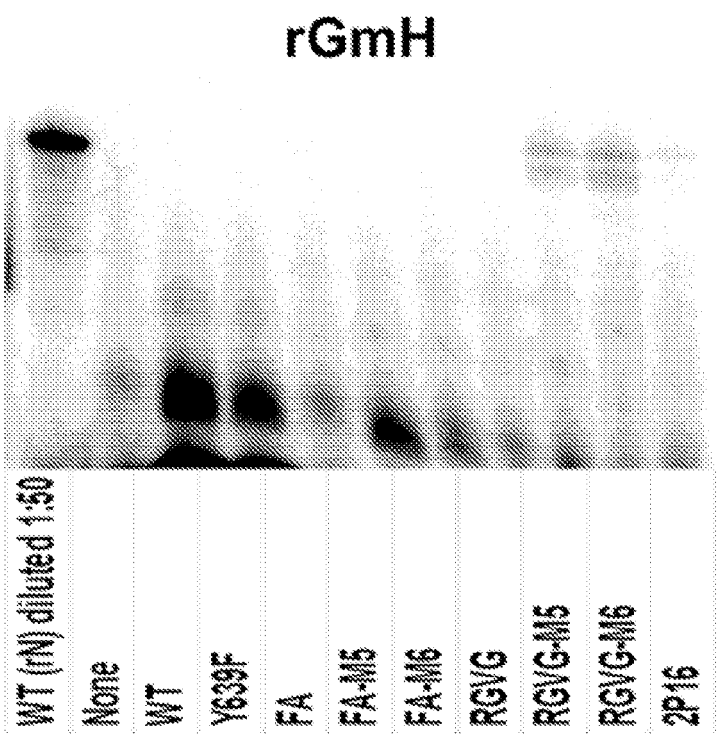
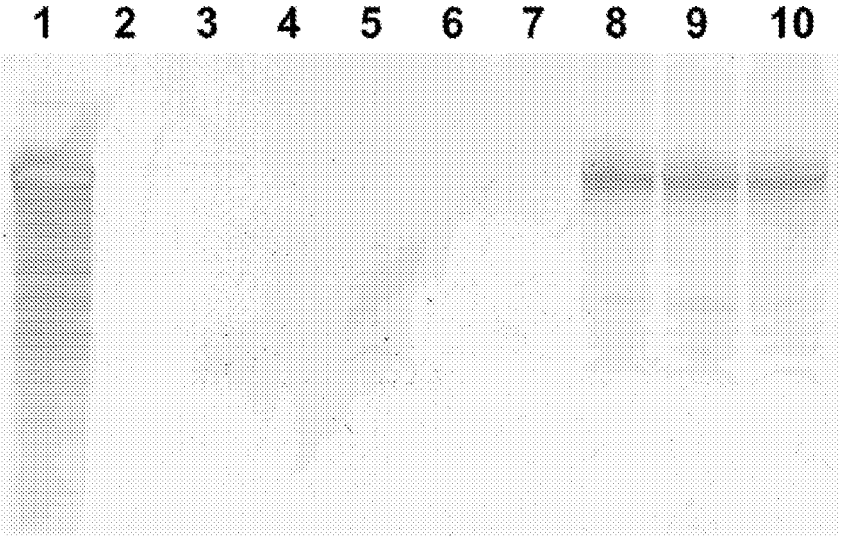

rGmH
WT (rN) diluted 1:50
None
WT
Y639F
FA
FA-M5
FA-M6
RGVG
RGVG-M5
RGVG-M6
2P16

FIG. 9



1
500 nM DNA
40 mM Tris-HCl pH 8.0
30 mM $MgCl_2$
6 mM spermidine
6 mM each NTP
10 mM DTT
500 nM RGVG-M6

2
500 nM DNA
40 mM Tris-HCl pH 8.0
30 mM $MgCl_2$
6 mM spermidine
6 mM each 2'-O-methyl-NTP
10 mM DTT
No RGVG-M6

3
500 nM DNA
40 mM Tris-HCl pH 8.0
30 mM $MgCl_2$
6 mM spermidine
6 mM each 2'-O-methyl-NTP
10 mM DTT
500 nM RGVG-M6

4
200 nM DNA
200mM HEPES pH 7.5
8 mM $MgCl_2$
2 mM spermidine
3.75 mM each 2'-O-methyl-NTP
40 mM DTT
0.01% Triton
200 nM RGVG-M6

5
200 nM DNA
200mM HEPES pH 7.5
8 mM $MgCl_2$
2 mM spermidine
3.75 mM each 2'-O-methyl-NTP
40 mM DTT
0.01% Triton
200 nM RGVG-M6

6
200 nM DNA
200mM HEPES pH 7.5
8 mM $MgCl_2$
2 mM spermidine
3.75 mM each 2'-O-methyl-NTP
40 mM DTT
0.01% Triton
10% PEG8000
200 nM RGVG-M6

7
200 nM DNA
200mM HEPES pH 7.5
8 mM $MgCl_2$
2 mM spermidine
3.75 mM each 2'-O-methyl-NTP
40 mM DTT
0.01% Triton
10% PEG8000
1.5 mM $MnCl_2$
200 nM RGVG-M6

8
200 nM DNA
200mM HEPES pH 7.5
5.5 mM $MgCl_2$
2 mM spermidine
0.5 mM each 2'-O-methyl-NTP
40 mM DTT
0.01% Triton
10% PEG8000
1.5 mM $MnCl_2$
10 U/ml YIPP
200 nM RGVG-M6

9
200 nM DNA
200mM HEPES pH 7.5
5.5 mM $MgCl_2$
2 mM spermidine
0.5 mM each 2'-O-methyl-NTP
40 mM DTT
0.01% Triton
10% PEG8000
1.5 mM $MnCl_2$
10 U/ml YIPP
0.5 mM GMP
200 nM RGVG-M6

10
200 nM DNA
200mM HEPES pH 7.5
5.5 mM $MgCl_2$
2 mM spermidine
0.5 mM each 2'-O-methyl-NTP
40 mM DTT
0.01% Triton
10% PEG8000
1.5 mM $MnCl_2$
10 U/ml YIPP
0.5 mM GMP
30 uM GTP
200 nM RGVG-M6

FIG. 10

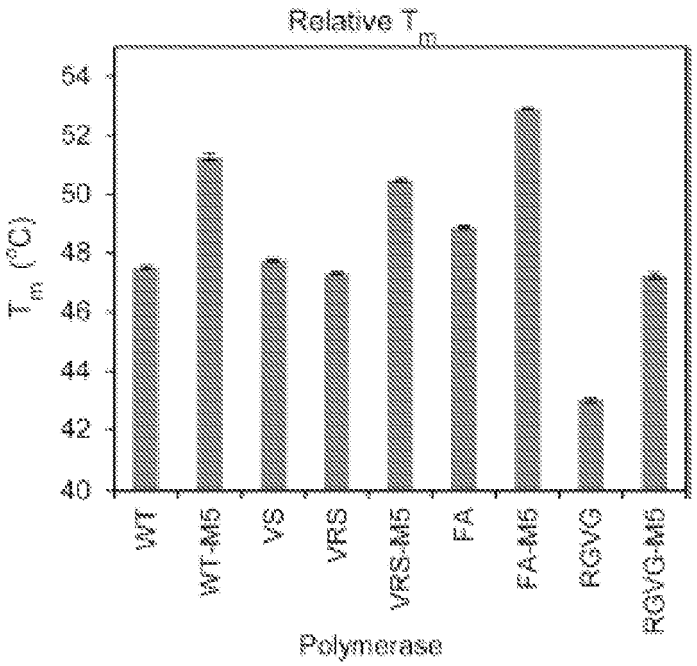


FIG. 11

Enzyme	WT	empty	WT	empty	WT	RGVG -M6	Y639L H784A	empty	RGVG -M5
NTPs	None		rN		mN	mN	mN		mN

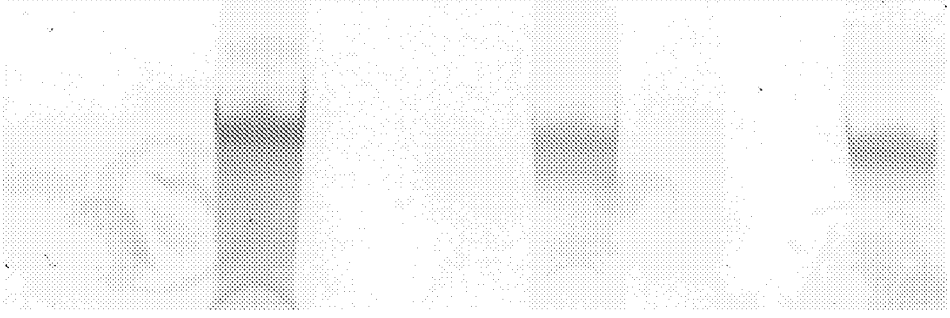


FIG. 12