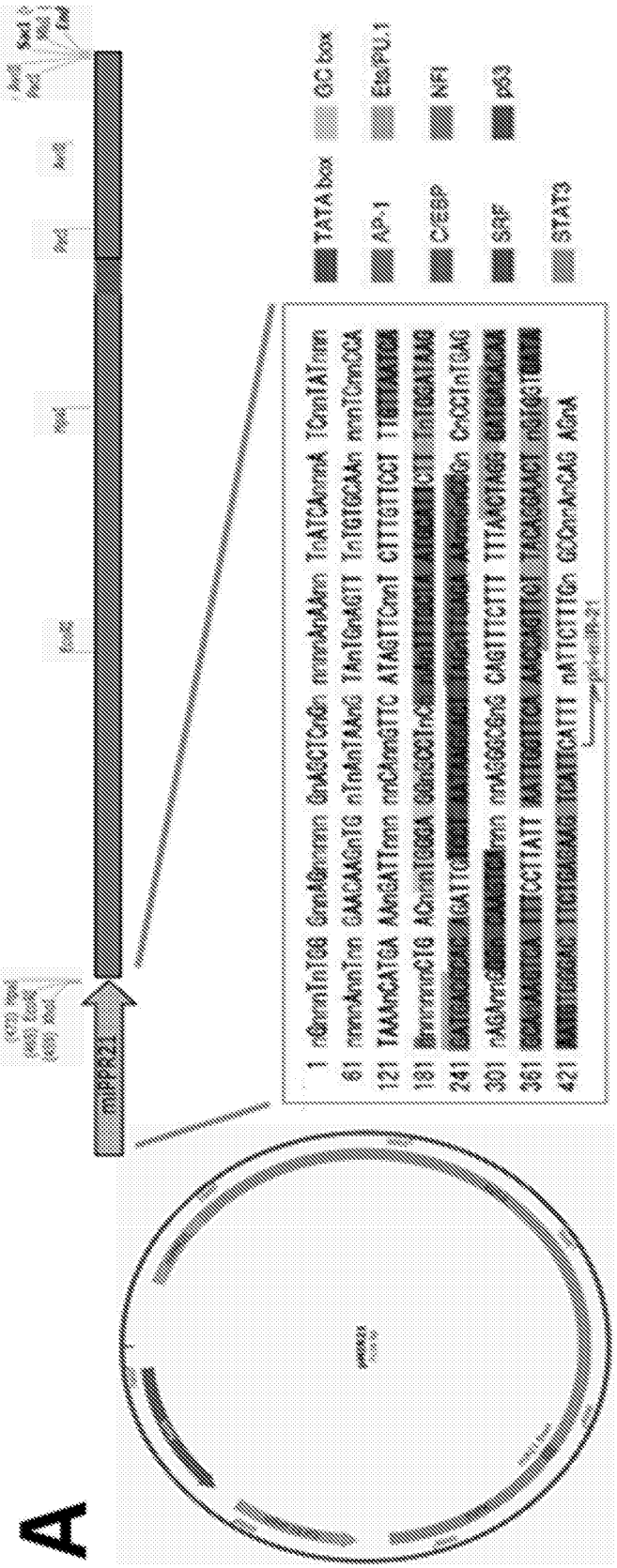

Figure 1A

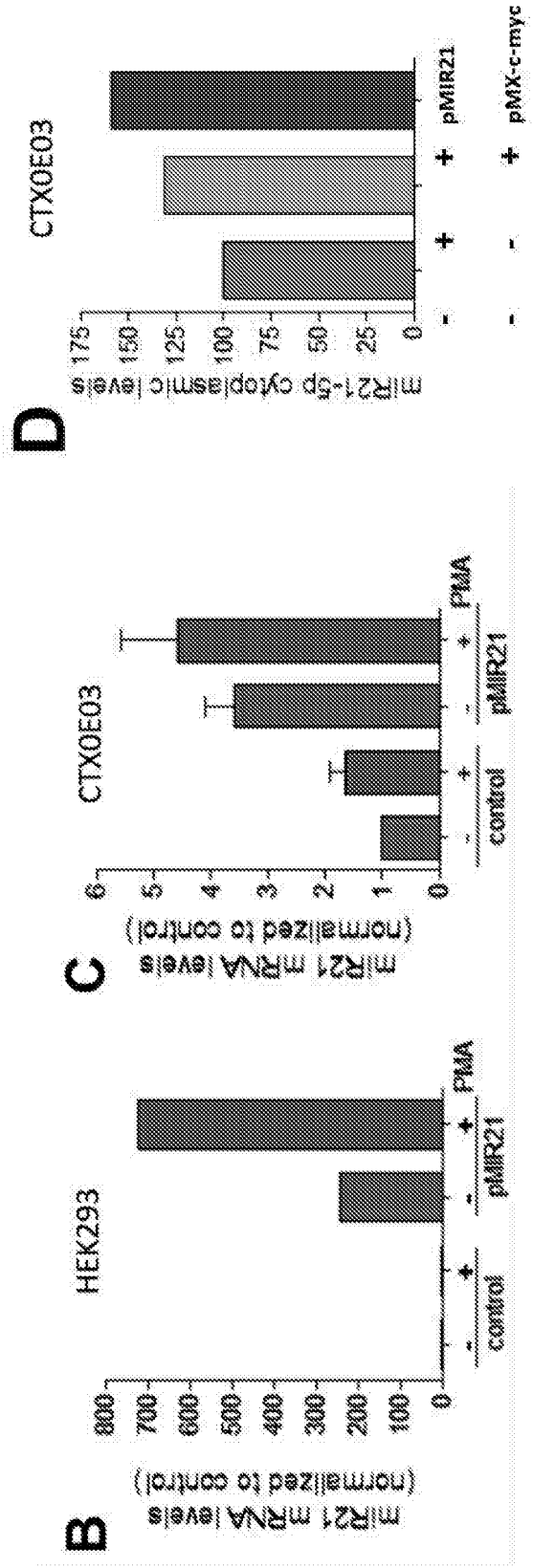
B
miR21 mRNA levels
(normalized to control)
HEK293
control
pMIR21
PMA
C
miR21 mRNA levels
(normalized to control)
CTX0E03
control
pMIR21
PMA
D
miR21-5p cytoplasmic levels
CTX0E03
pMIR21
pMX-c-myc

Figure 1B – 1D

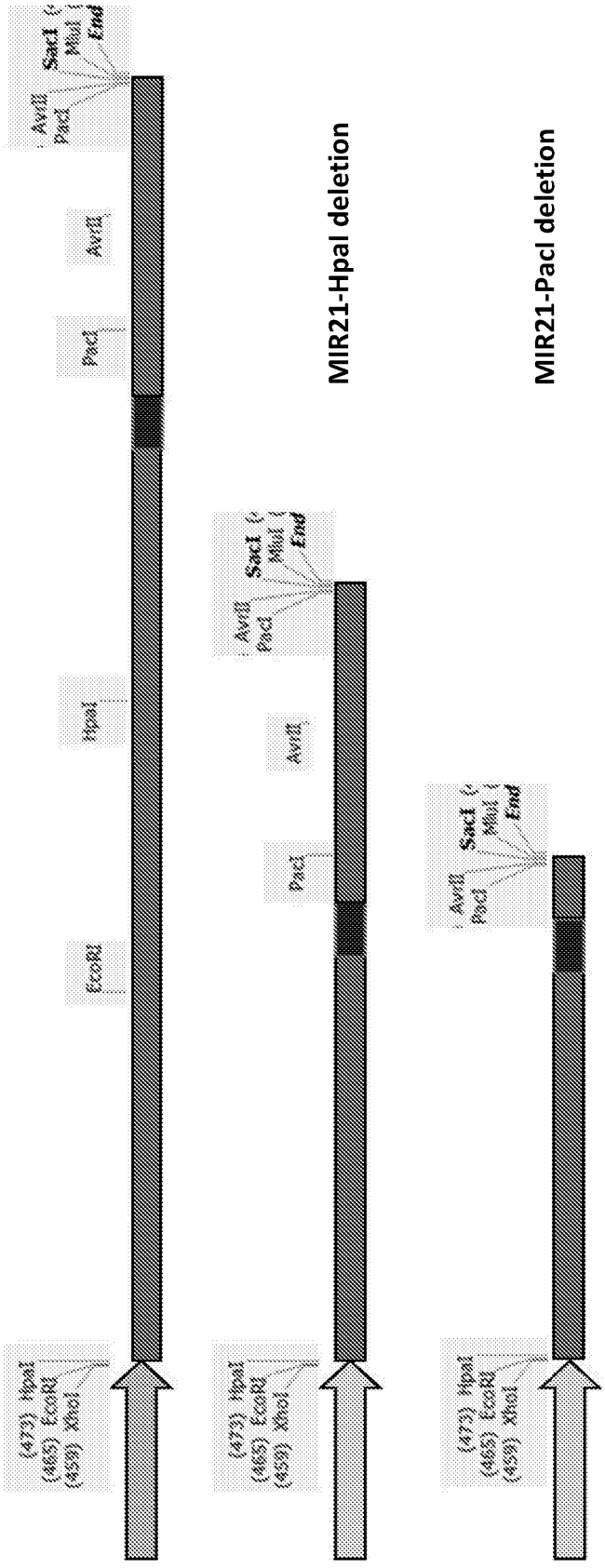
MIR21-HpaI deletion
MIR21-PacI deletion
SacI
End
AvrII
PacI
HpaI
EcoRI
XhoI
(473)
(465)
(459)

Figure 2A

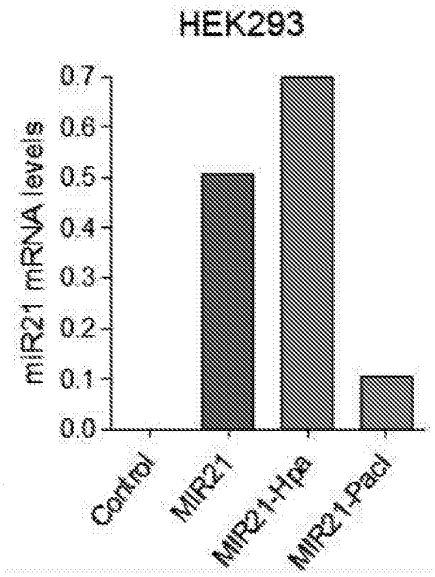
HEK293
miR21 mRNA levels
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
MIR21
MIR21-Hpa
MIR21-Pacl

Figure 2B

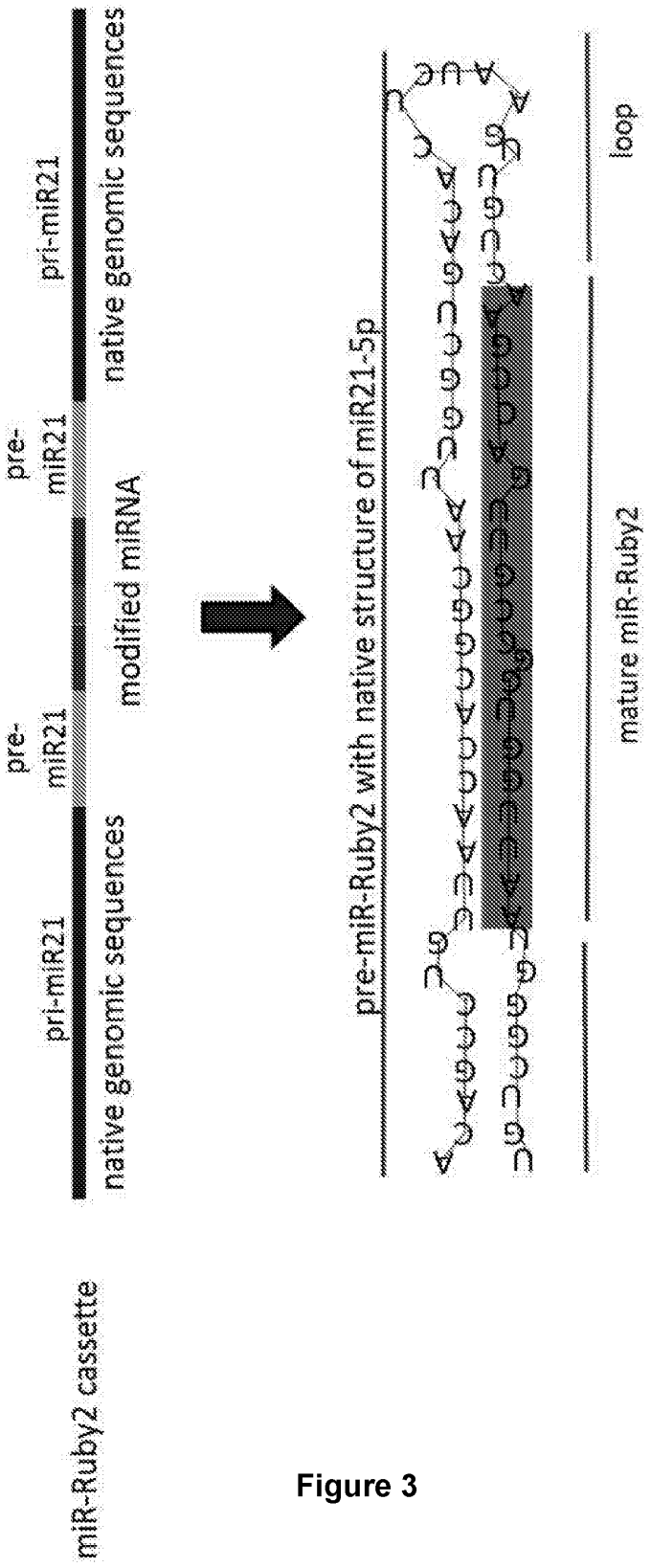
miR-Ruby2 cassette
native genomic sequences
pri-miR21
pre-miR21
modified miRNA
native genomic sequences
pri-miR21
pre-miR21
pre-miR-Ruby2 with native structure of miR21-5p
loop
mature miR-Ruby2

Figure 3

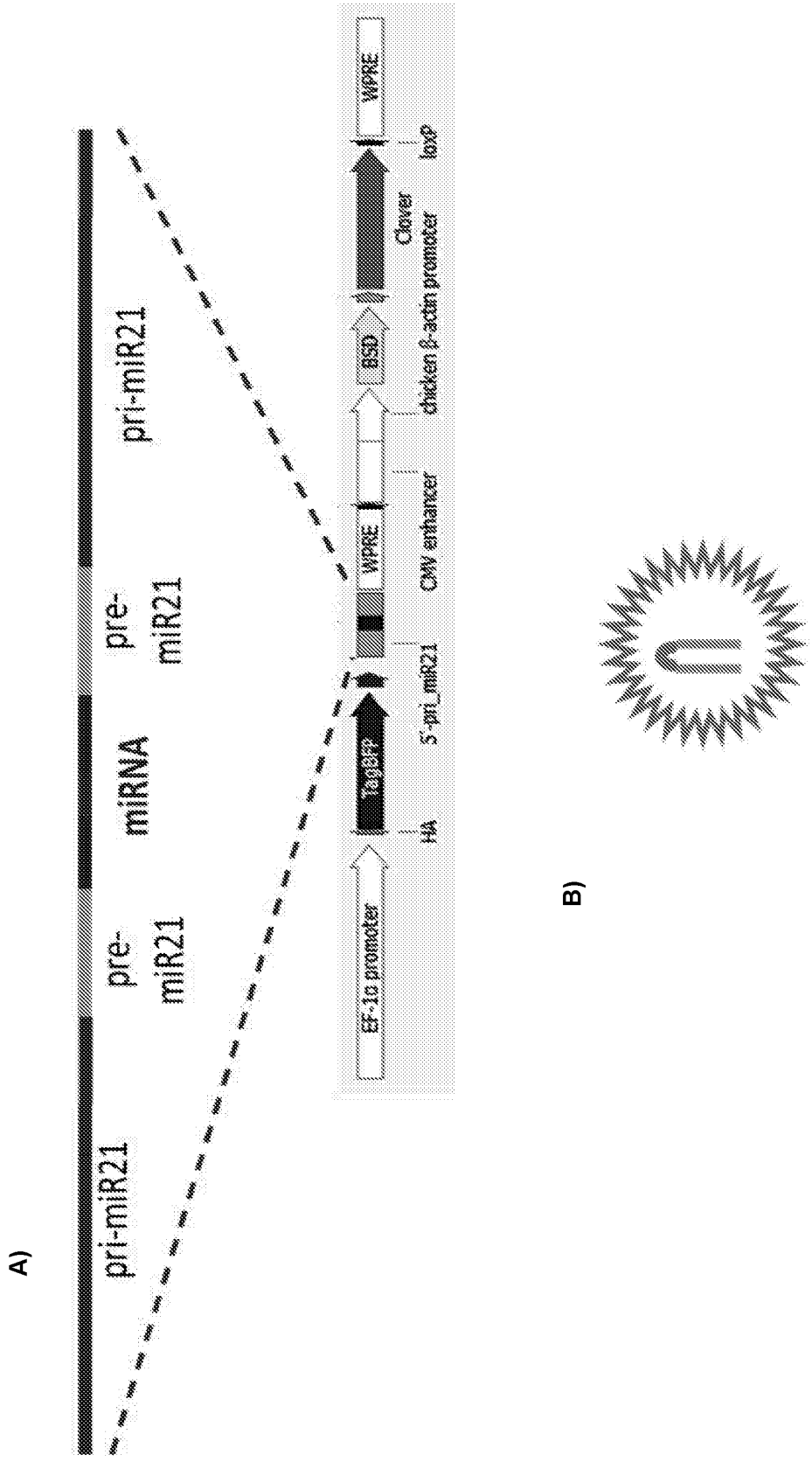
A)
pri-miR21
pre-miR21
miRNA
pre-miR21
pri-miR21
B)
EF-1α promoter
HA
TagBFP
5'-pri_miR21
WPRE
CMV enhancer
BSD
chicken β-actin promoter
Clover
loxP
WPRE

Figure 4

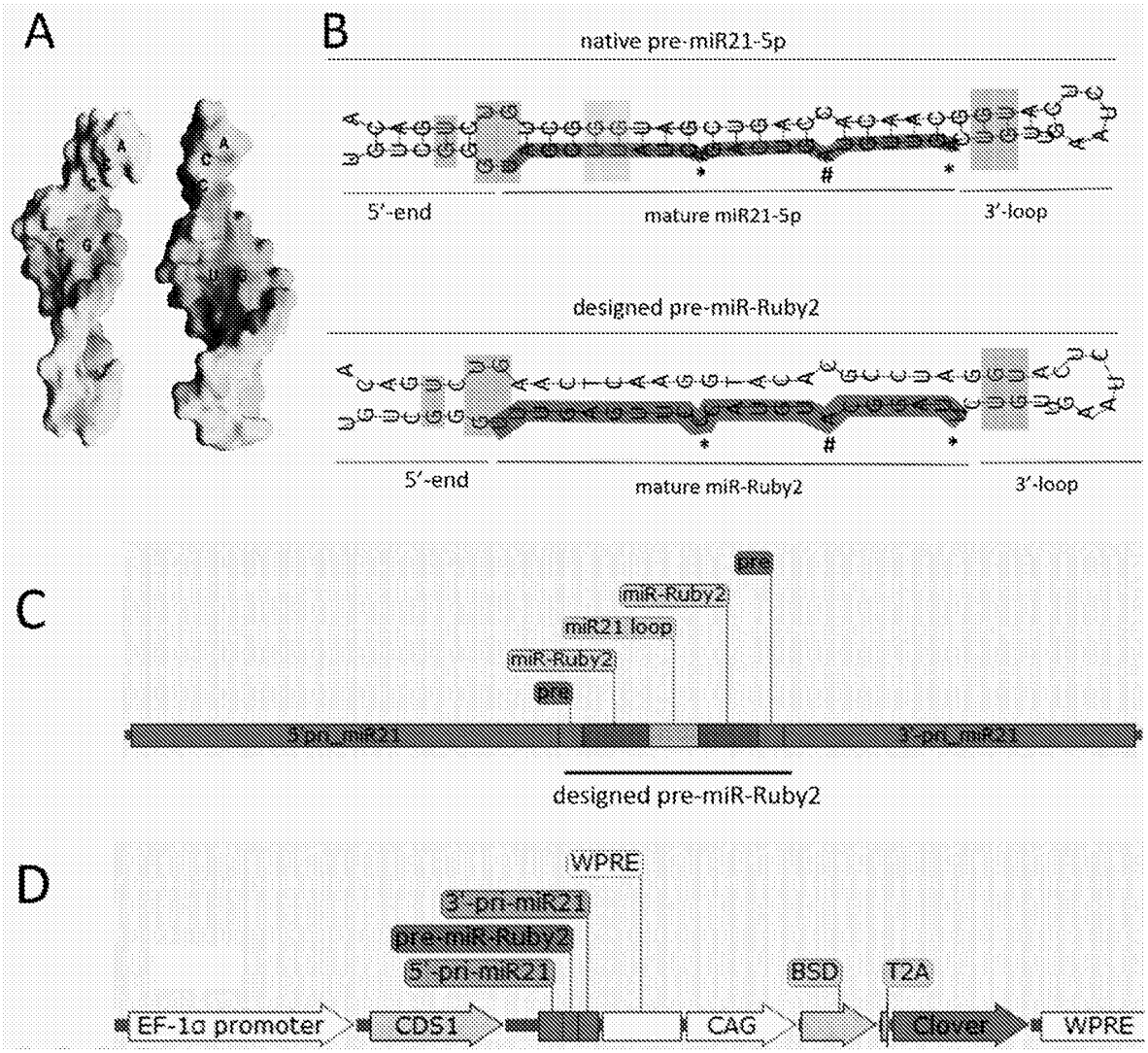
A
B
native pre-miR21-5p
5'-end
mature miR21-5p
3'-loop
designed pre-miR-Ruby2
5'-end
mature miR-Ruby2
3'-loop
C
pre
miR-Ruby2
miR21 loop
miR-Ruby2
pre
5'pri_miR21
3'pri_miR21
designed pre-miR-Ruby2
D
WPRE
3'-pri-miR21
pre-miR-Ruby2
5'-pri-miR21
BSD
T2A
EF-1α promoter
CDS1
CAG
Clover
WPRE

Figure 5

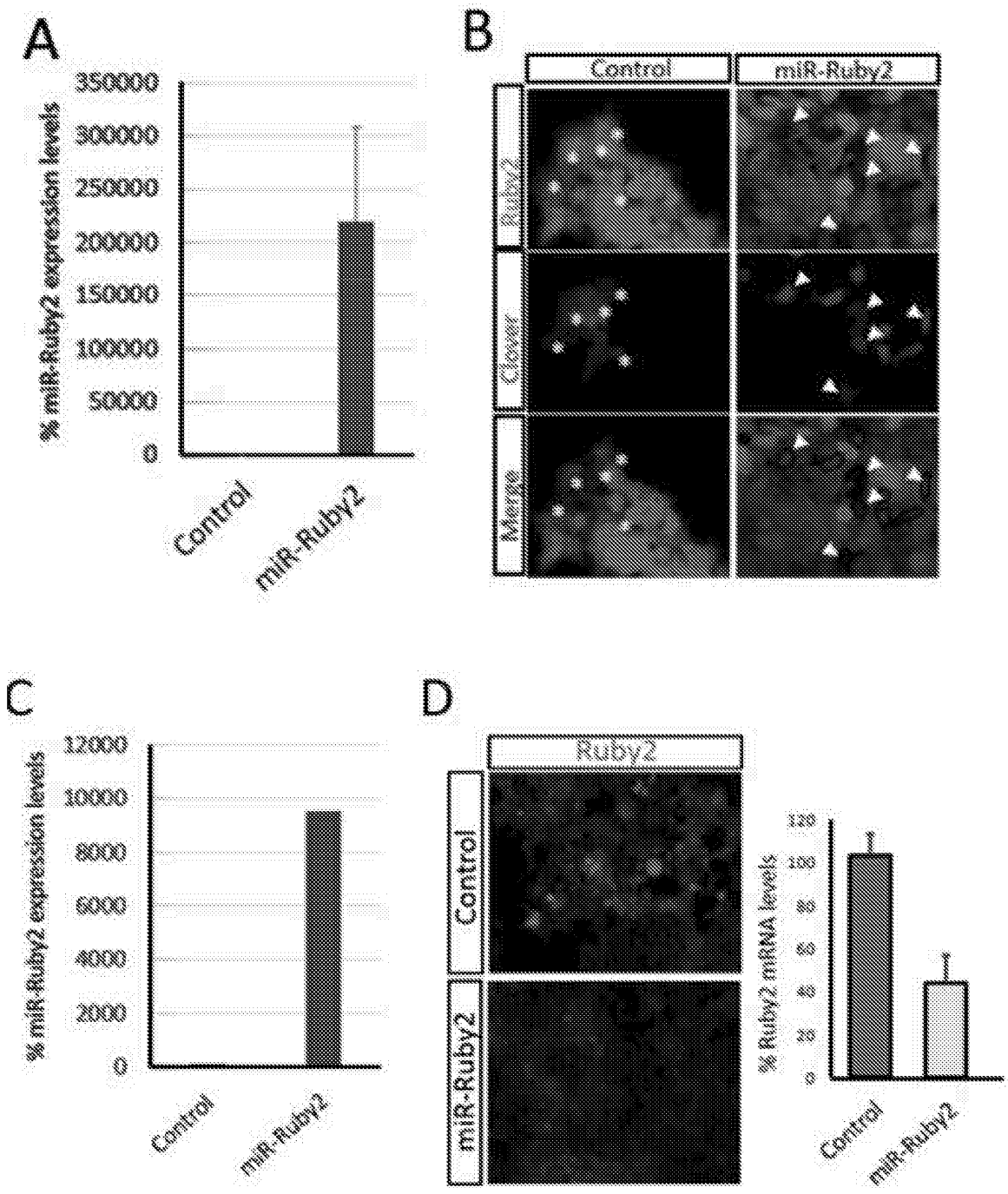
A
350000
300000
250000
200000
150000
100000
50000
0
% miR-Ruby2 expression levels
Control
miR-Ruby2
B
Control
miR-Ruby2
Ruby2
Clover
Merge
C
12000
10000
8000
6000
4000
2000
0
% miR-Ruby2 expression levels
Control
miR-Ruby2
D
Ruby2
Control
miR-Ruby2
% Ruby2 mRNA levels
Control
miR-Ruby2

Figure 6