Figure 1

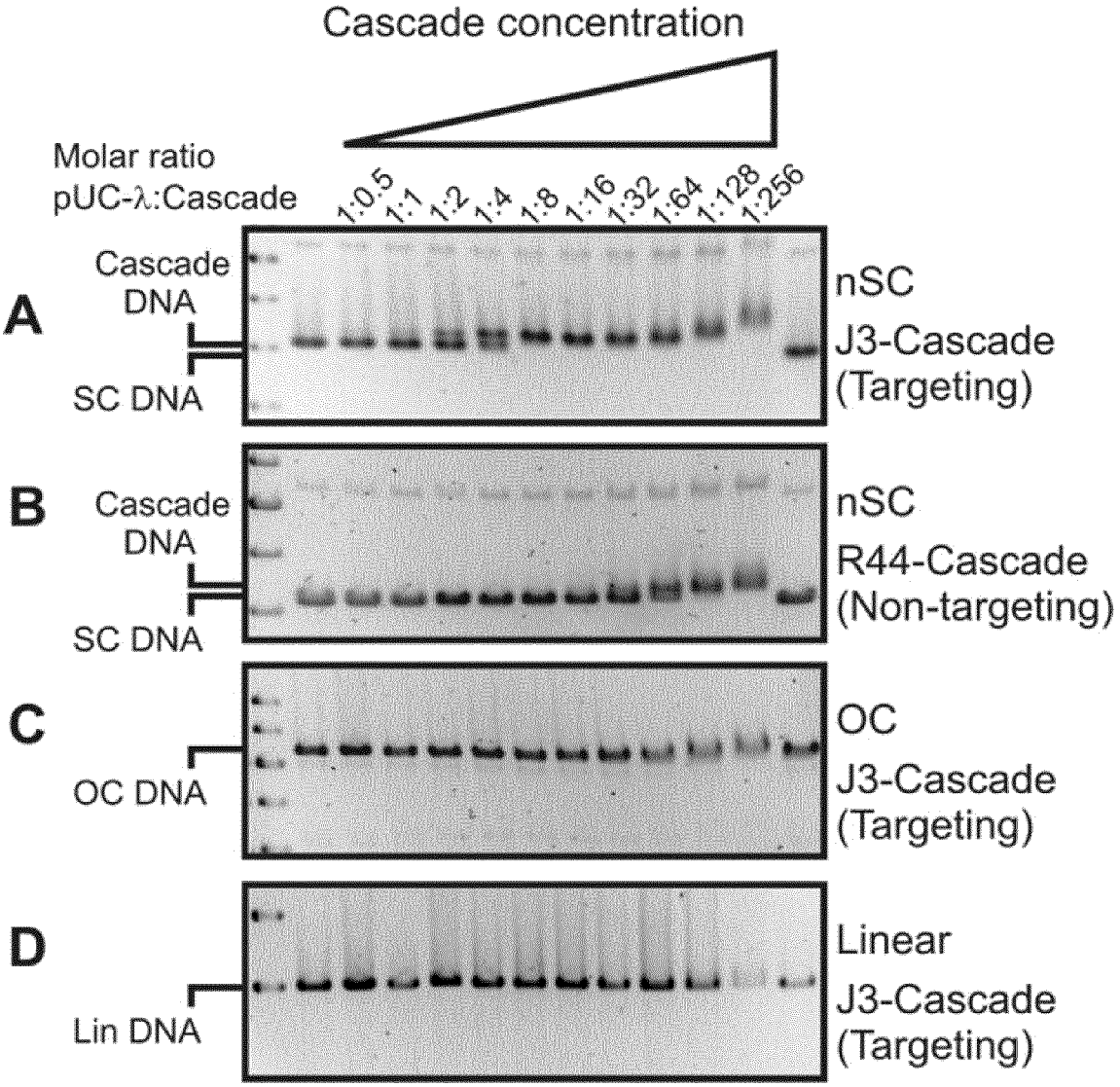

Cascade concentration
Molar ratio
pUC-λ:Cascade
1:0.5
1:1
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
A
Cascade DNA
SC DNA
nSC
J3-Cascade (Targeting)
B
Cascade DNA
SC DNA
nSC
R44-Cascade (Non-targeting)
C
OC DNA
OC
J3-Cascade (Targeting)
D
Lin DNA
Linear
J3-Cascade (Targeting)

Figure 1 continued

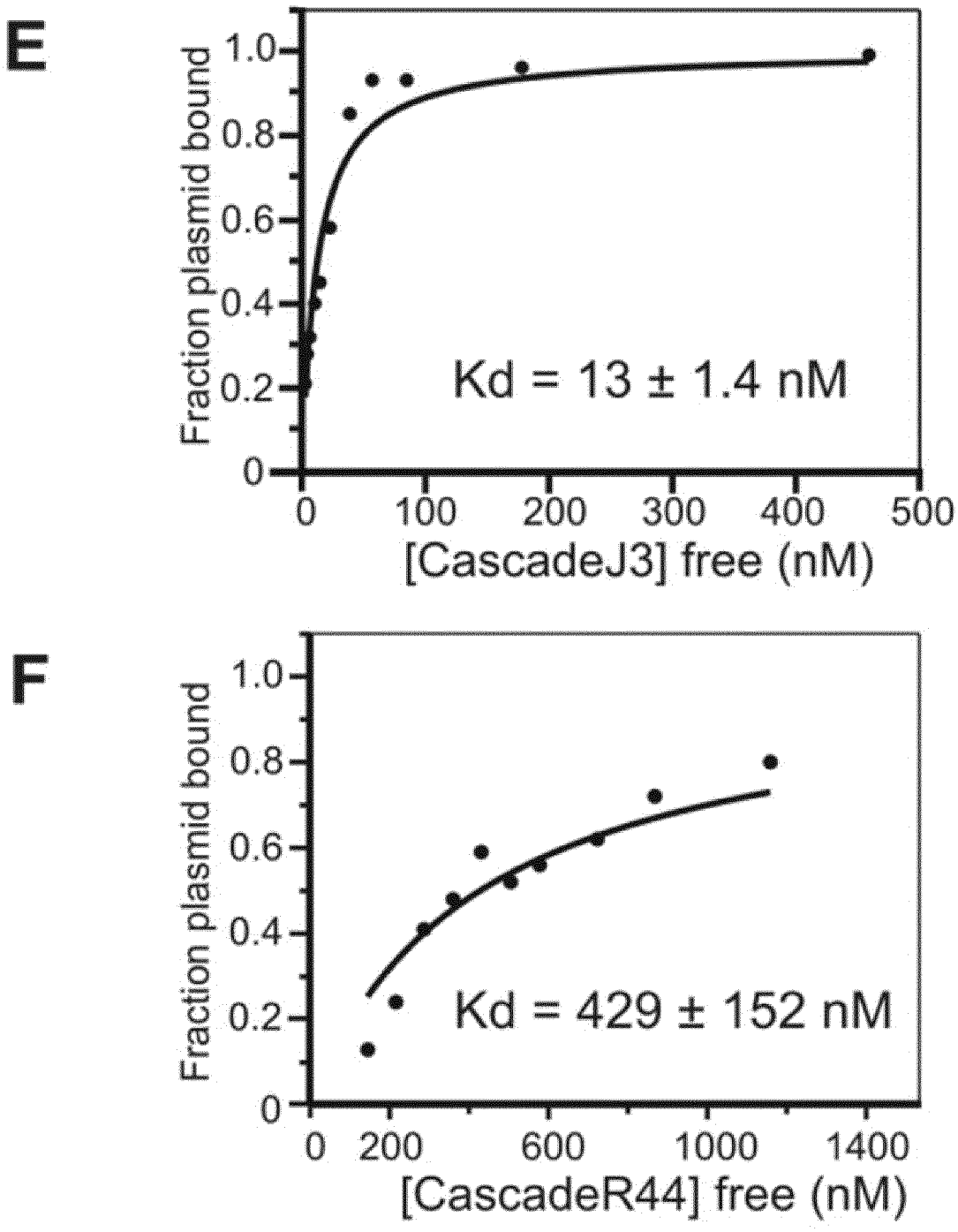


Figure 1 continued

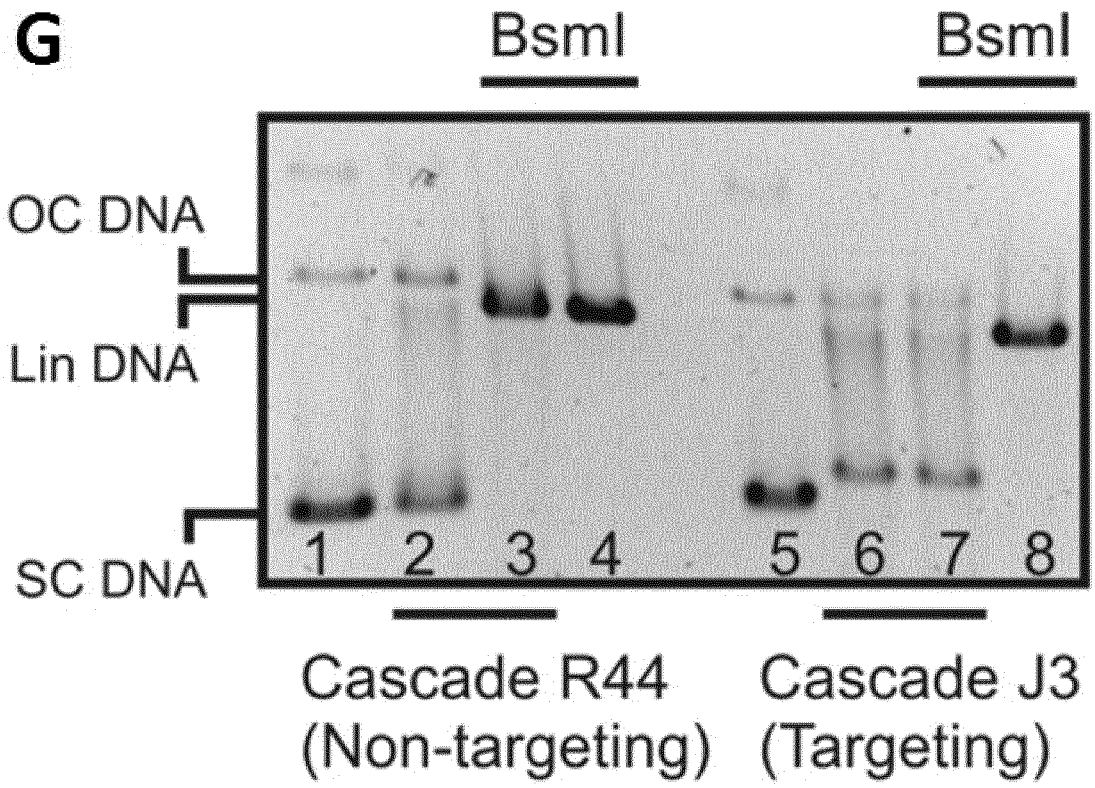


Figure 1 continued

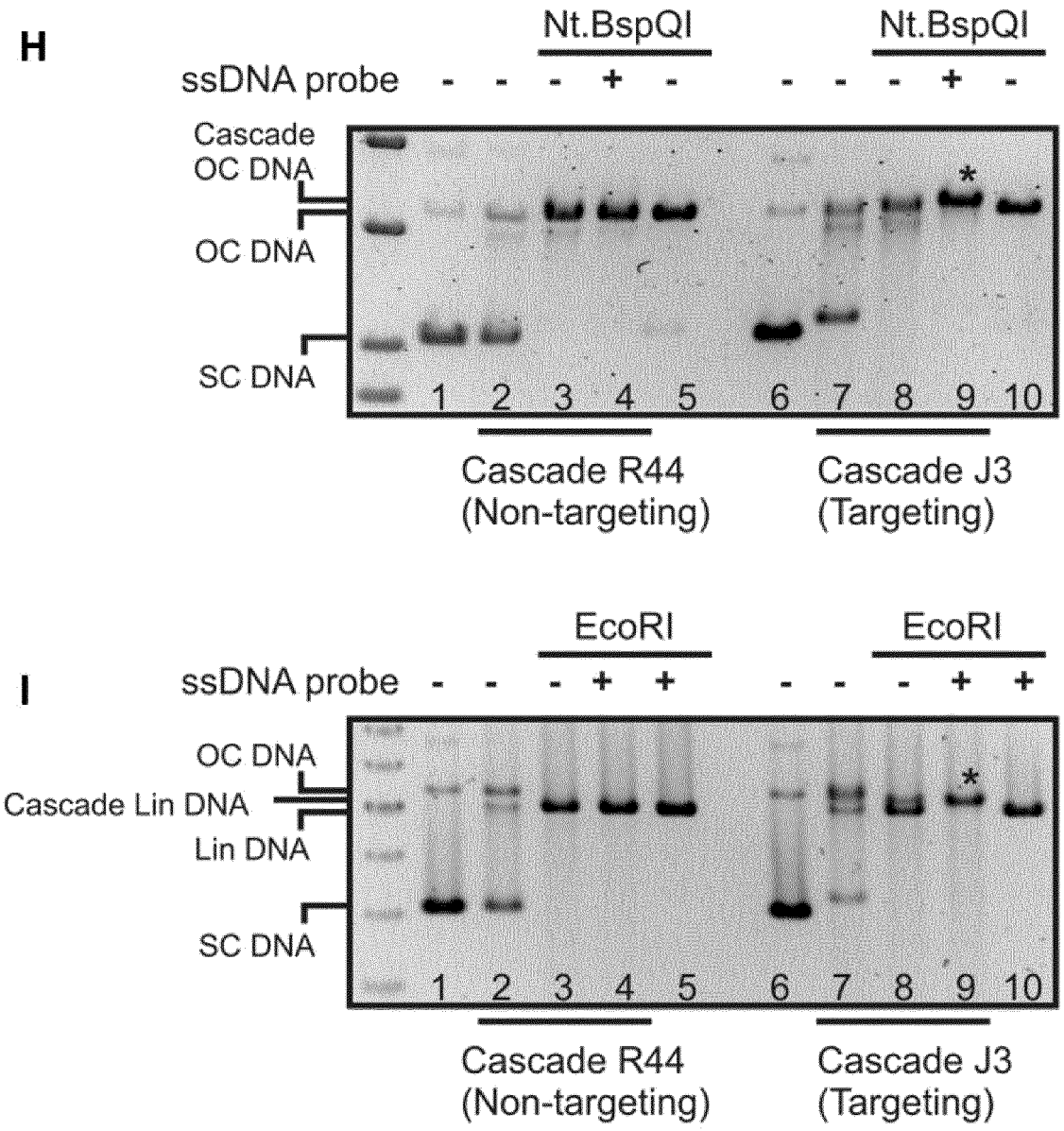


Figure 2

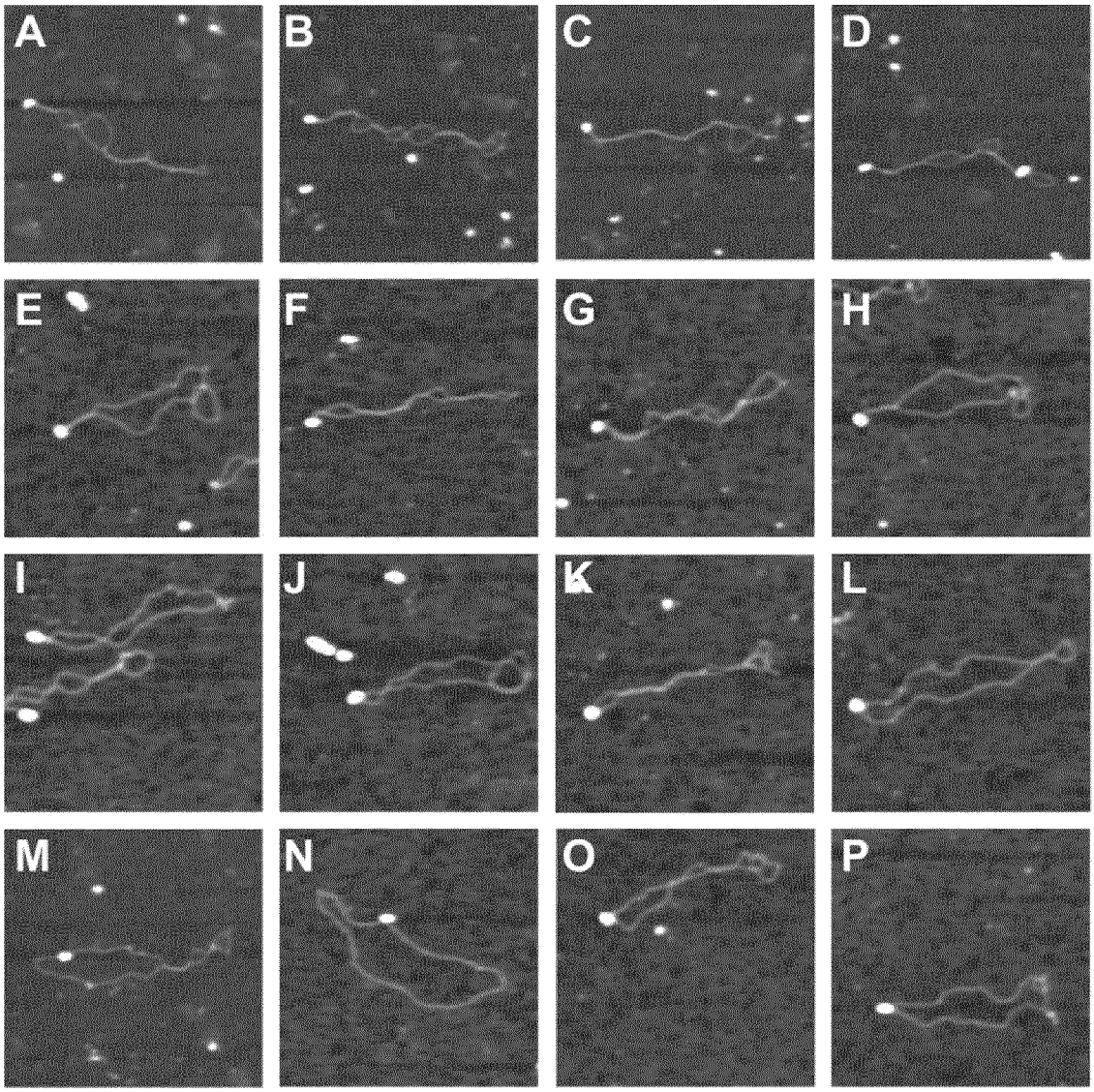


Figure 3

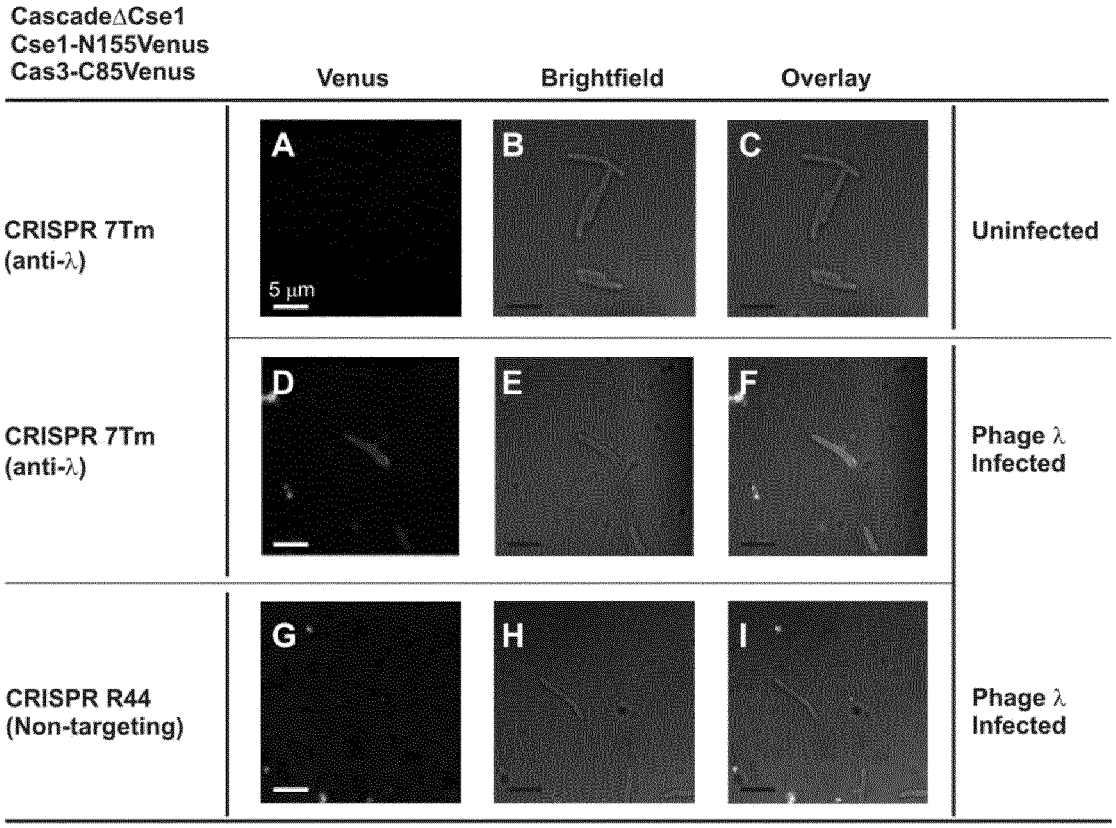

CascadeΔCse1
Cse1-N155Venus
Cas3-C85Venus
Venus
Brightfield
Overlay
A
B
C
5 µm
CRISPR 7Tm
(anti-λ)
Uninfected
D
E
F
CRISPR 7Tm
(anti-λ)
Phage λ
Infected
G
H
I
CRISPR R44
(Non-targeting)
Phage λ
Infected

Figure 3 continued

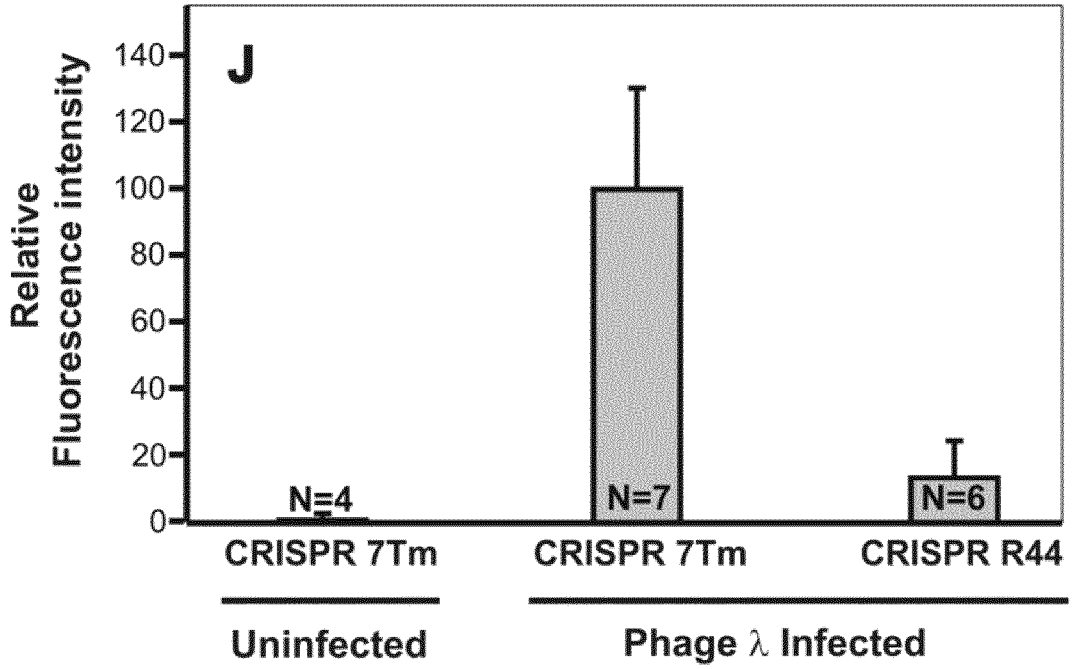


Figure 4

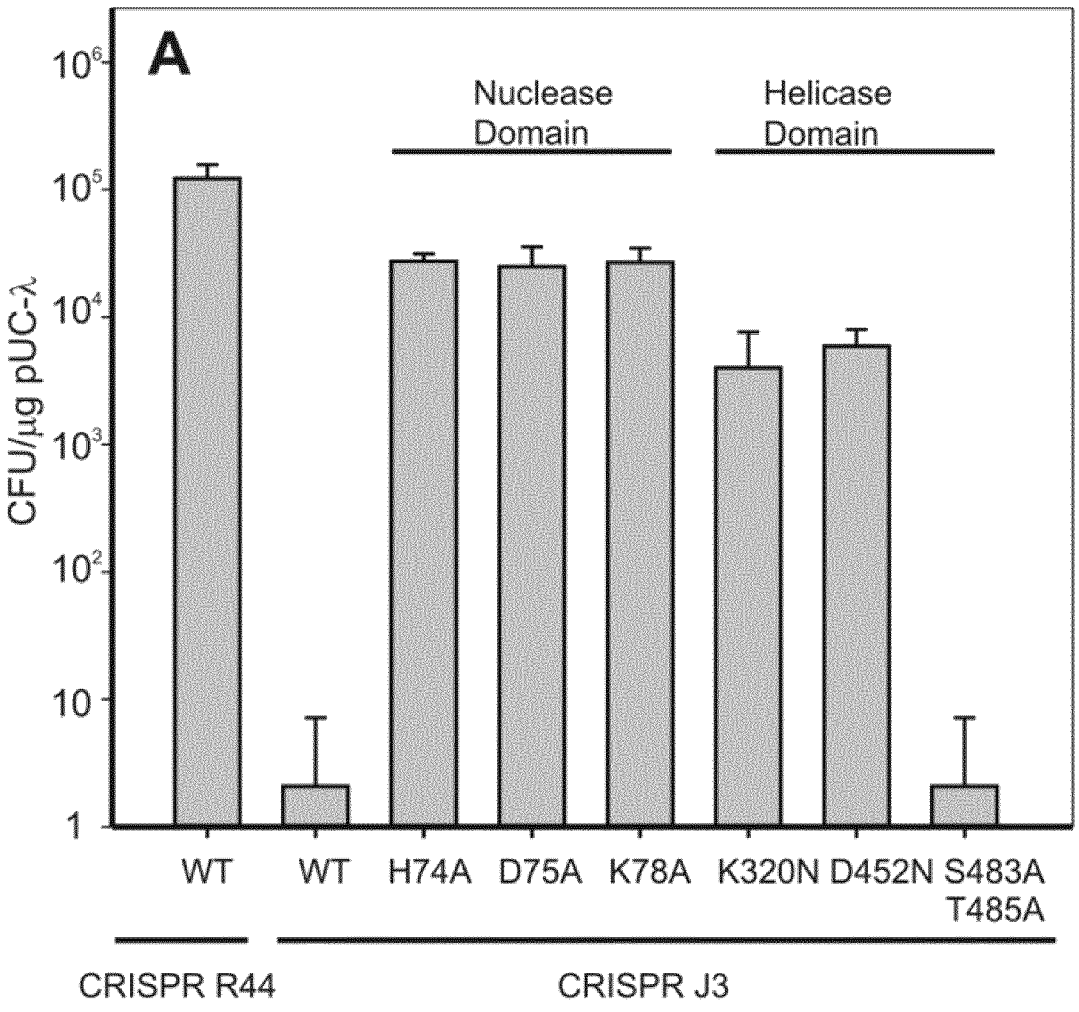

A
Nuclease Domain
Helicase Domain
CFU/µg pUC-λ
10^6
10^5
10^4
10^3
10^2
10
1
WT
WT
H74A
D75A
K78A
K320N
D452N
S483A T485A
CRISPR R44
CRISPR J3

Figure 4 continued

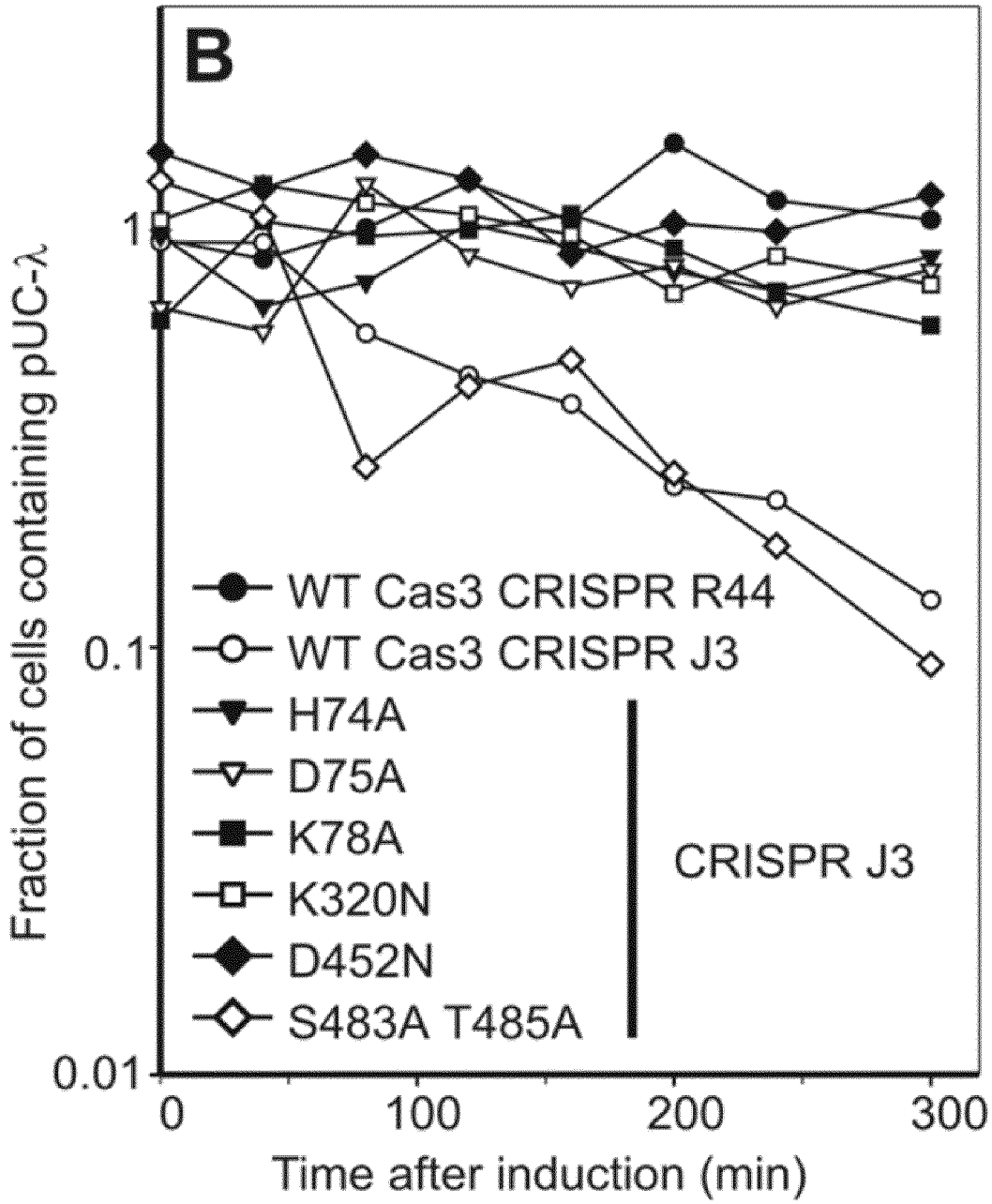


Figure 5

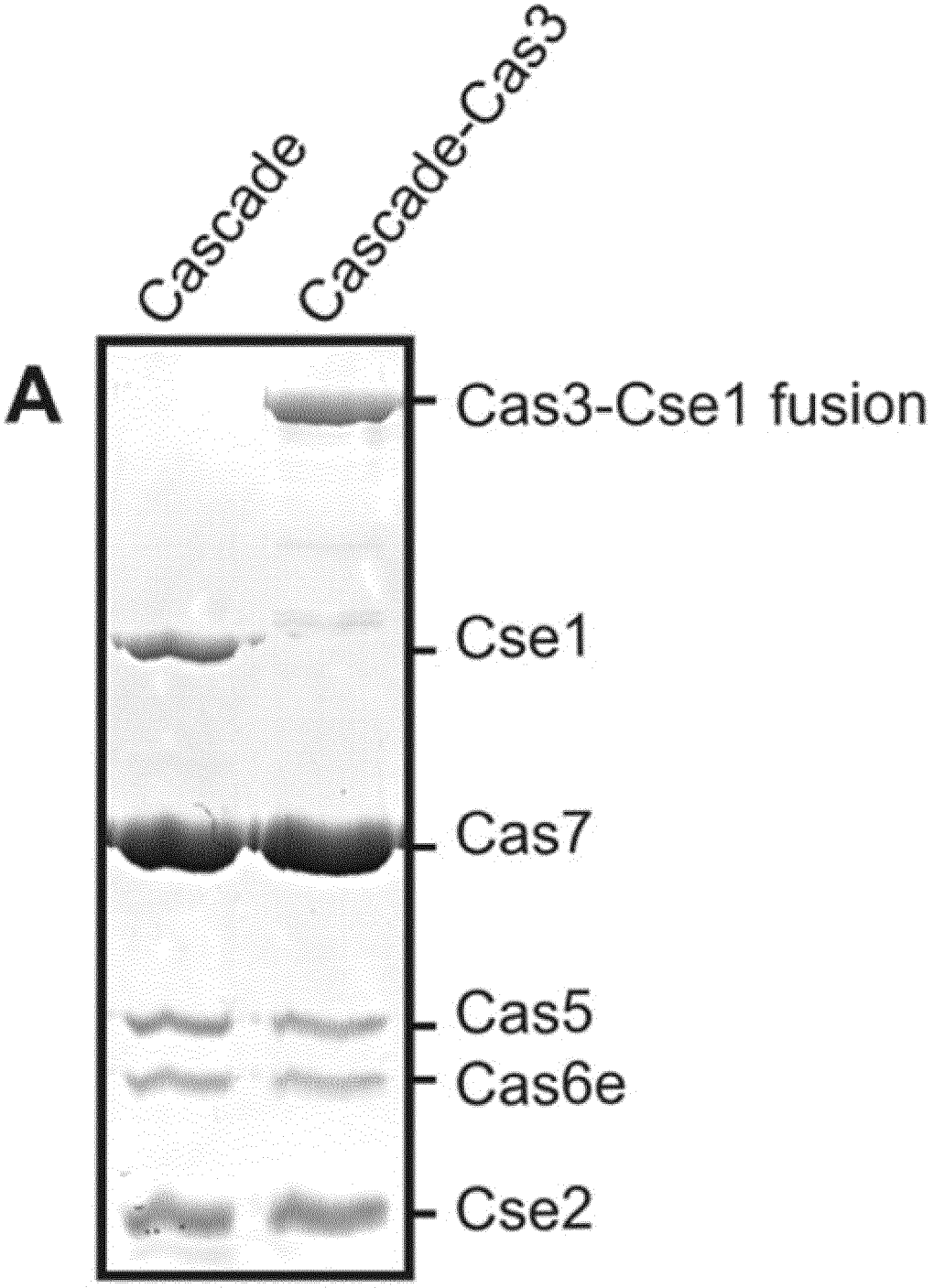

Cascade
Cascade-Cas3
A
Cas3-Cse1 fusion
Cse1
Cas7
Cas5
Cas6e
Cse2

Figure 5 continued

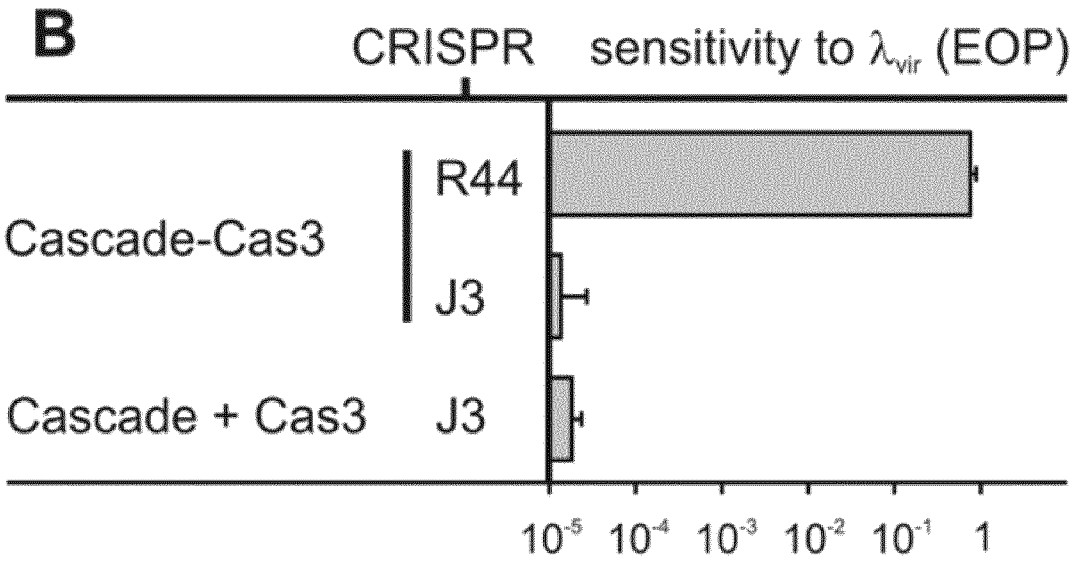


Figure 5 continued

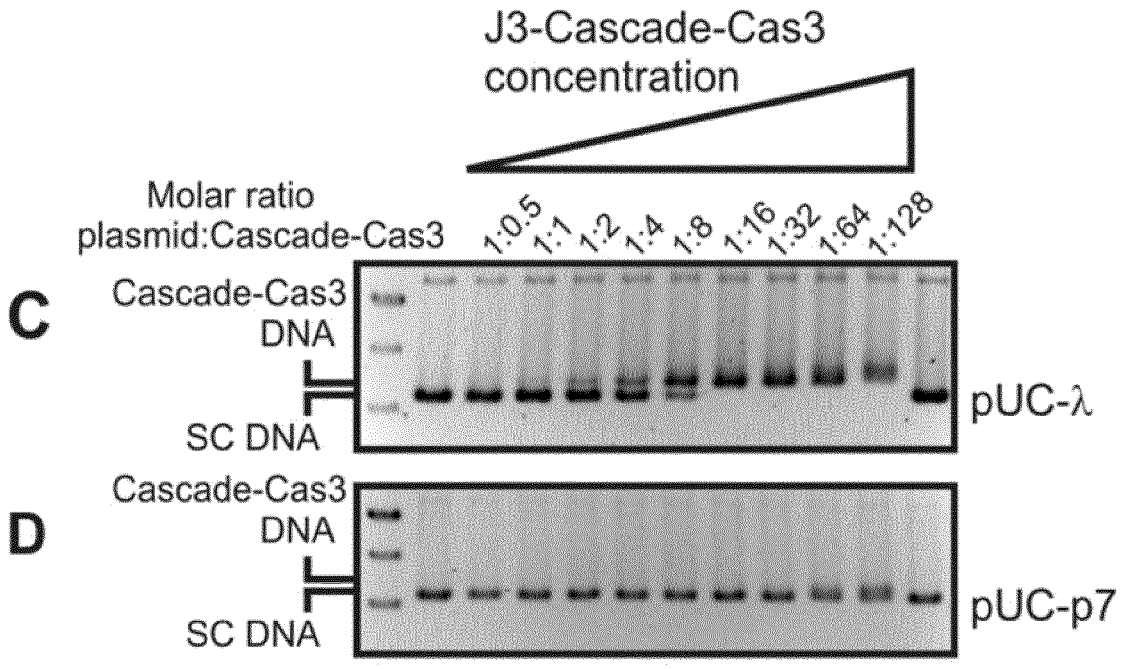


Figure 5 continued

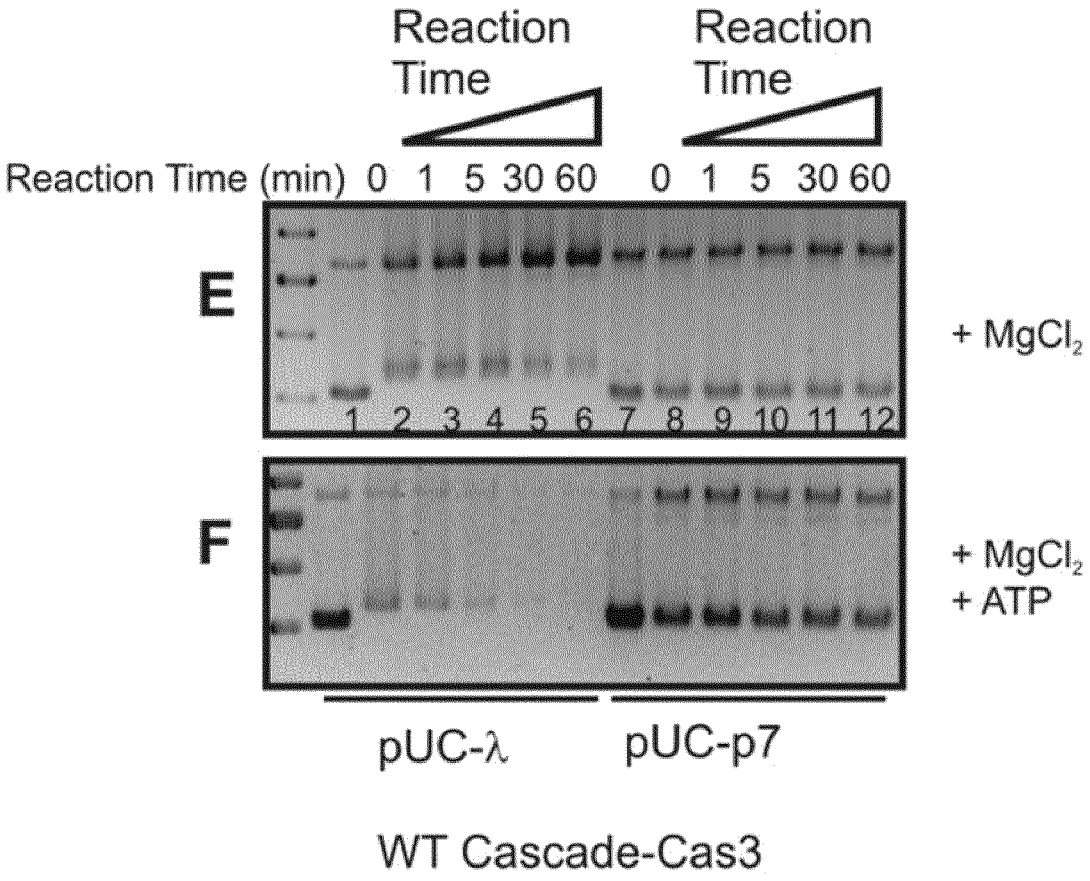


Figure 5 continued

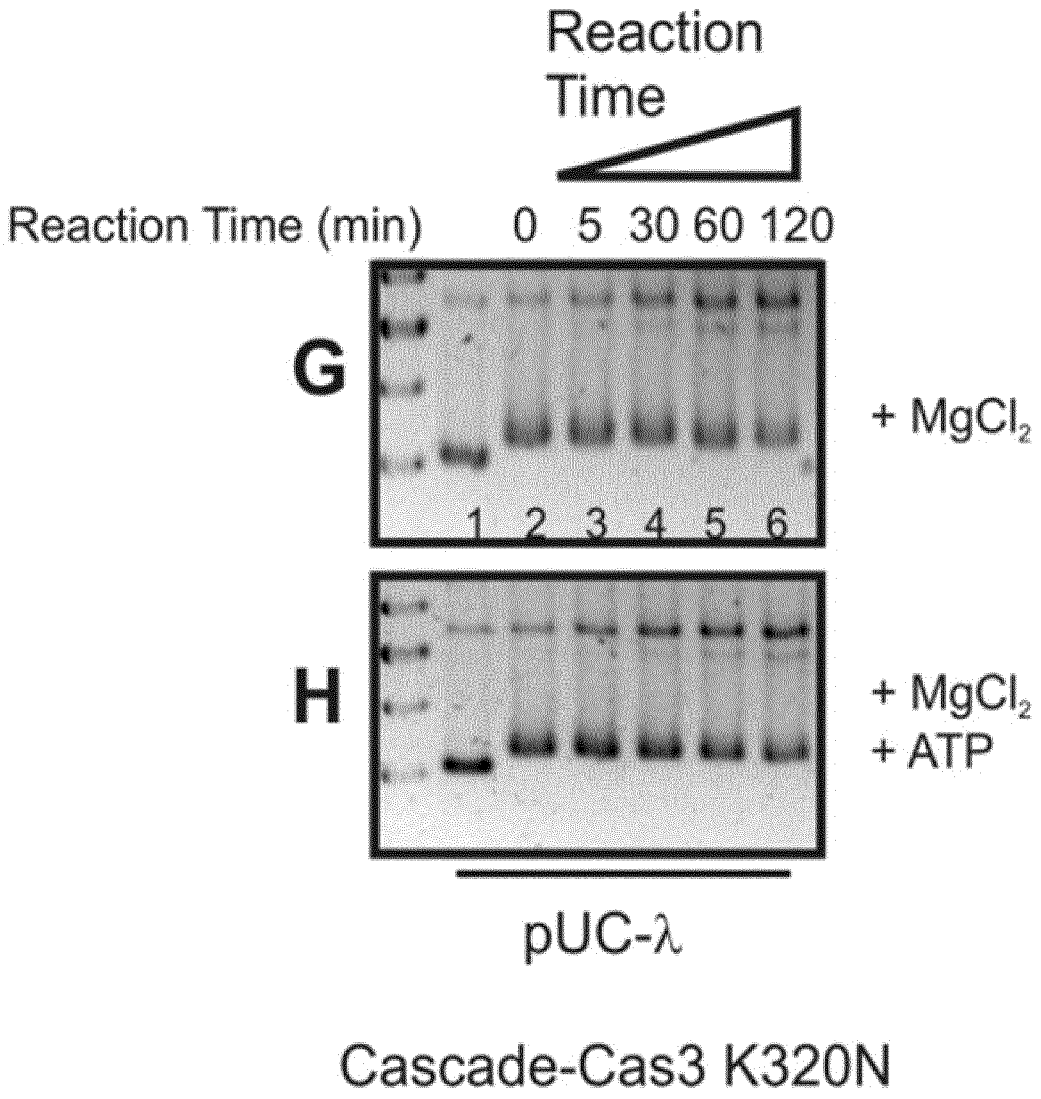


Figure 6

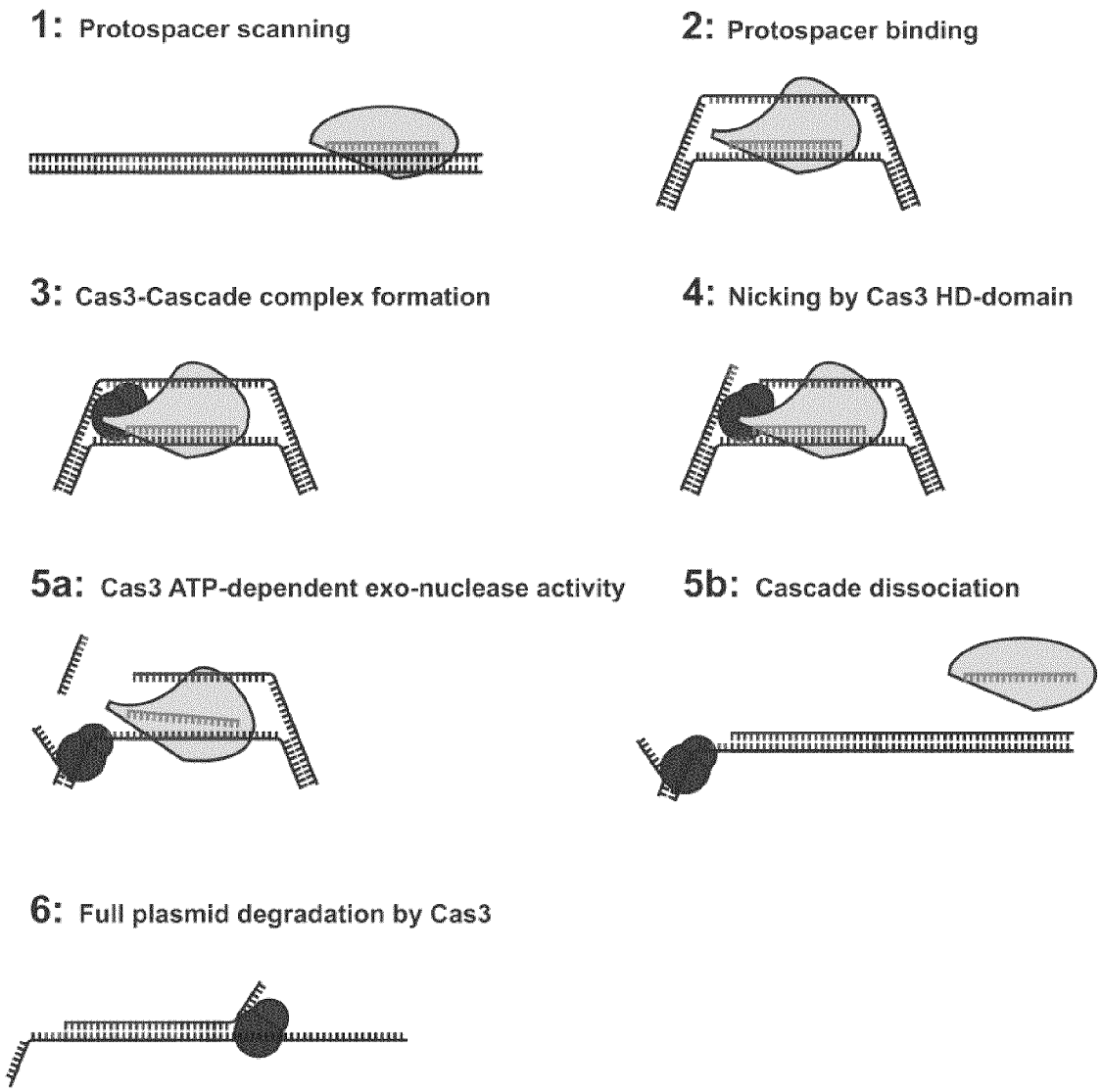

1: Protospacer scanning
2: Protospacer binding
3: Cas3-Cascade complex formation
4: Nicking by Cas3 HD-domain
5a: Cas3 ATP-dependent exo-nuclease activity
5b: Cascade dissociation
6: Full plasmid degradation by Cas3

Figure 7

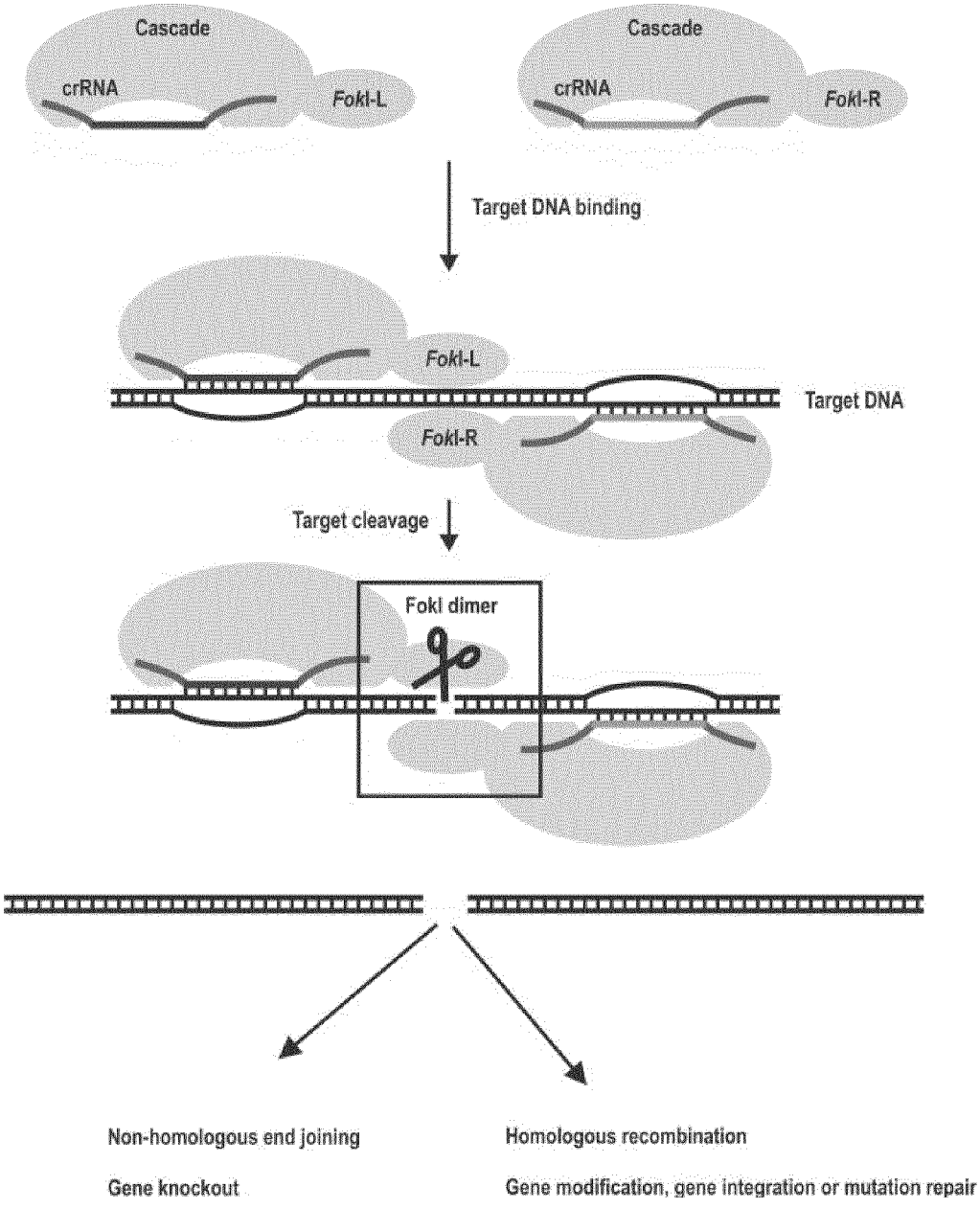

Cascade
crRNA
FokI-L
Cascade
crRNA
FokI-R
Target DNA binding
FokI-L
Target DNA
FokI-R
Target cleavage
FokI dimer
Non-homologous end joining
Gene knockout
Homologous recombination
Gene modification, gene integration or mutation repair

Figure 8

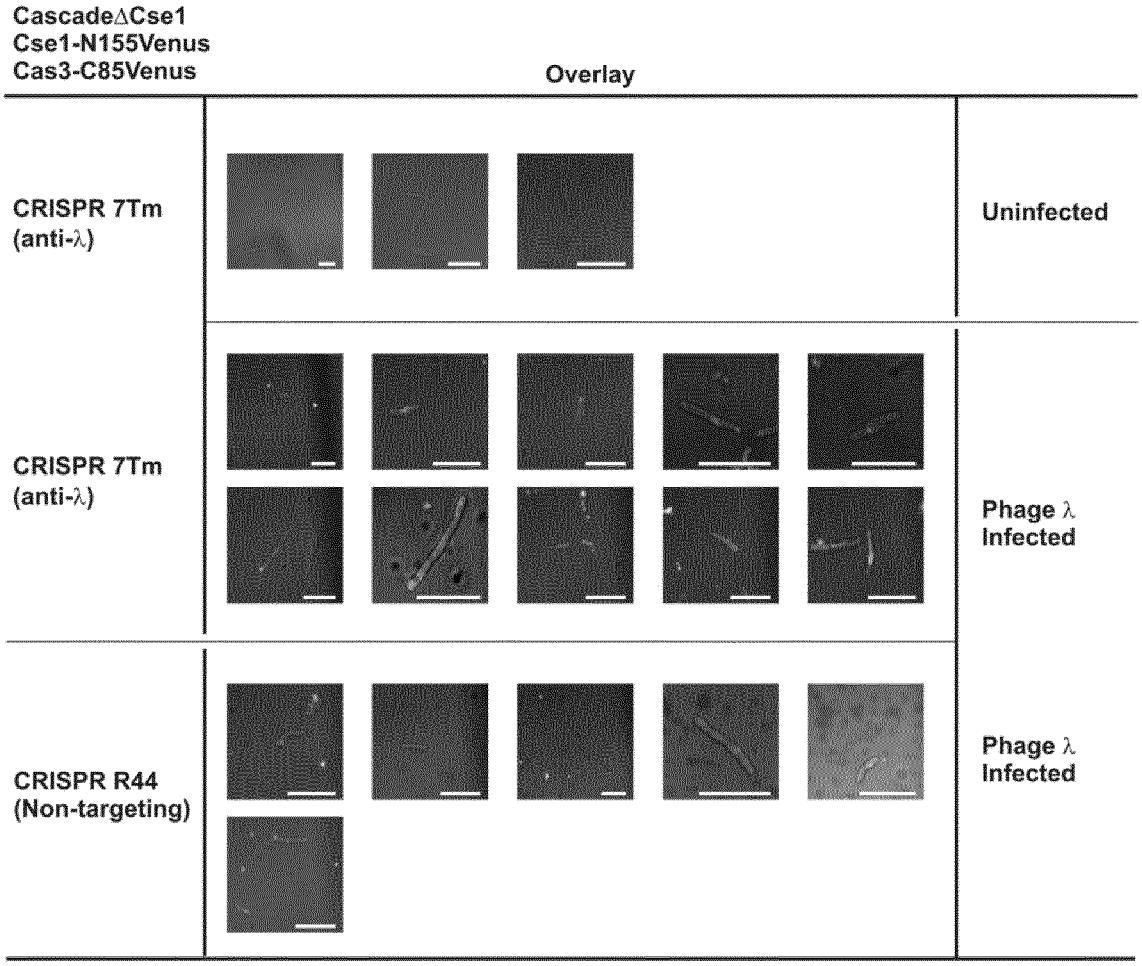

CascadeΔCse1
Cse1-N155Venus
Cas3-C85Venus
Overlay
CRISPR 7Tm
(anti-λ)
Uninfected
CRISPR 7Tm
(anti-λ)
Phage λ
Infected
CRISPR R44
(Non-targeting)
Phage λ
Infected

Figure 9

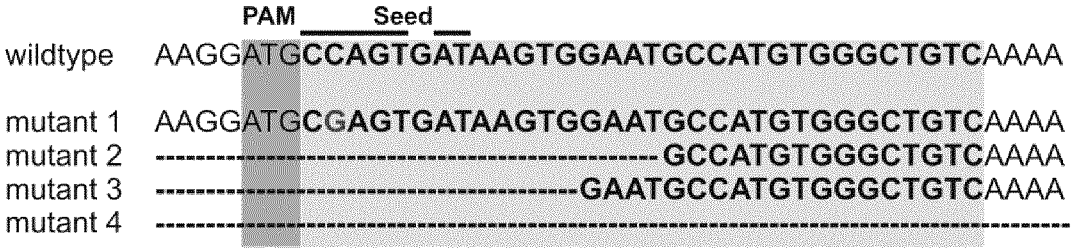

PAM
Seed
wildtype AAGGATGCCAGTGATAAGTGGAATGCCATGTGGGCTGTCAAAA
mutant 1 AAGGATGCGAGTGATAAGTGGAATGCCATGTGGGCTGTCAAAA
mutant 2 ----------GCCATGTGGGCTGTCAAAA
mutant 3 ----------GAATGCCATGTGGGCTGTCAAAA
mutant 4 ----------

Figure 10

Figure 10 continued



Figure 11

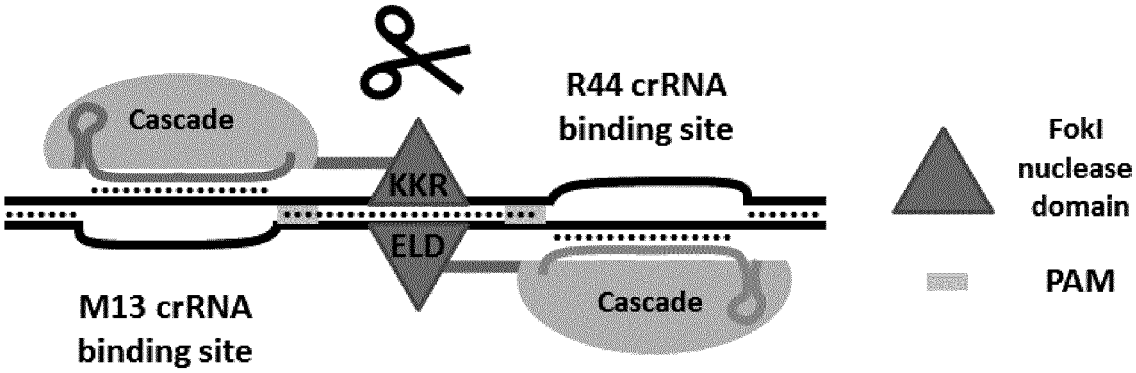

Cascade
R44 crRNA
binding site
KKR
ELD
M13 crRNA
binding site
Cascade
FokI
nuclease
domain
PAM

Figure 12

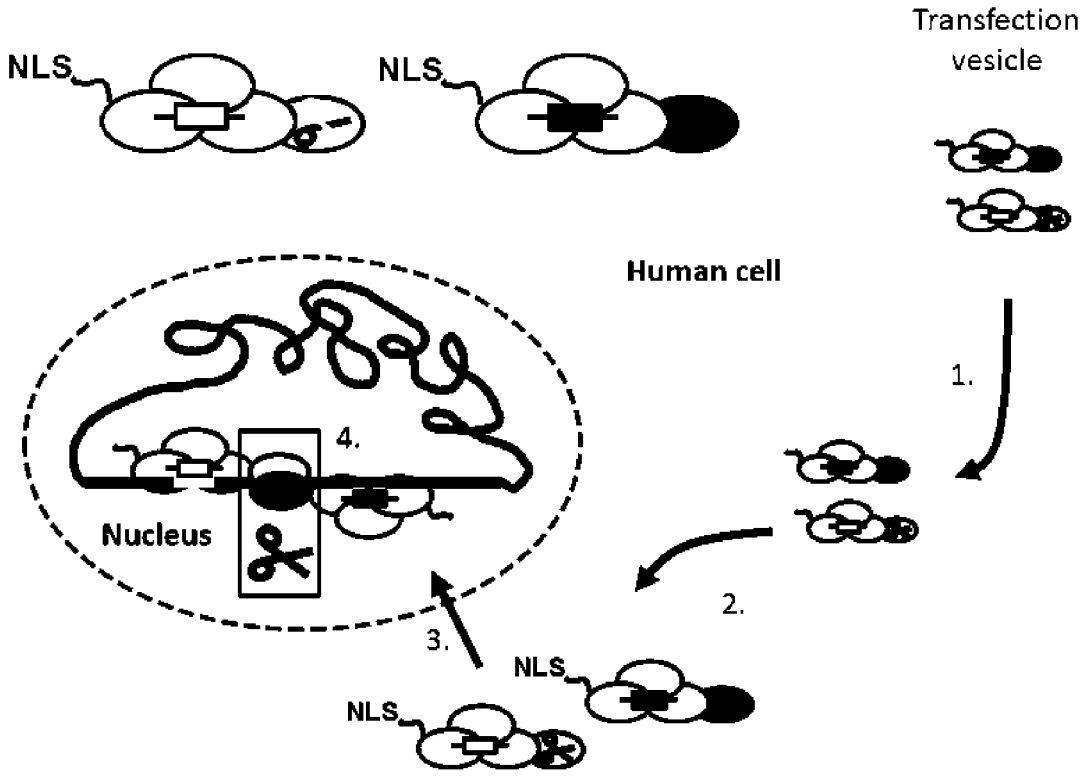

NLS
NLS
Transfection vesicle
Human cell
1.
2.
3.
4.
Nucleus
NLS
NLS

Figure 13

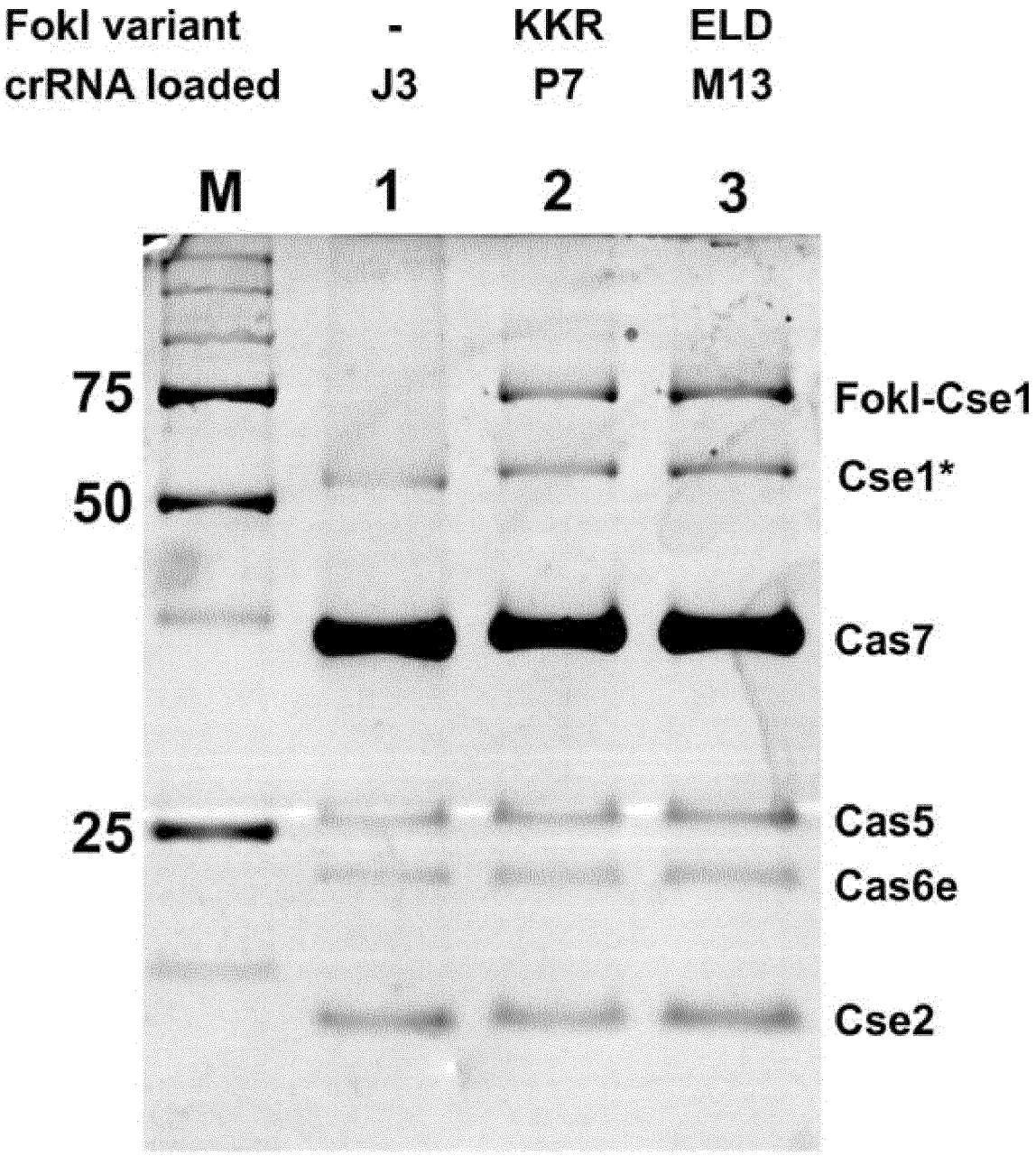

FokI variant
-
KKR
ELD
crRNA loaded
J3
P7
M13
M
1
2
3
75
50
25
FokI-Cse1
Cse1*
Cas7
Cas5
Cas6e
Cse2

Figure 14

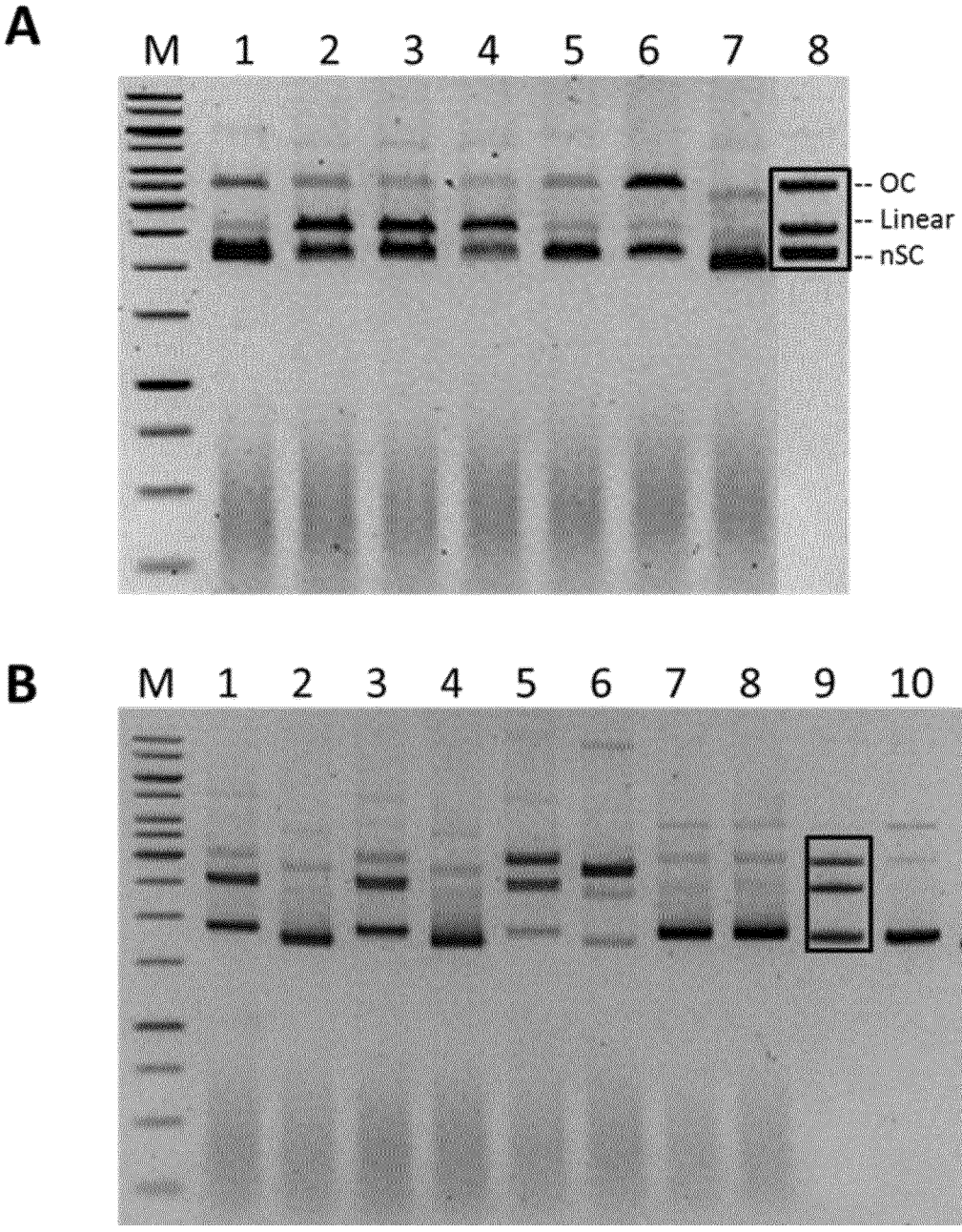
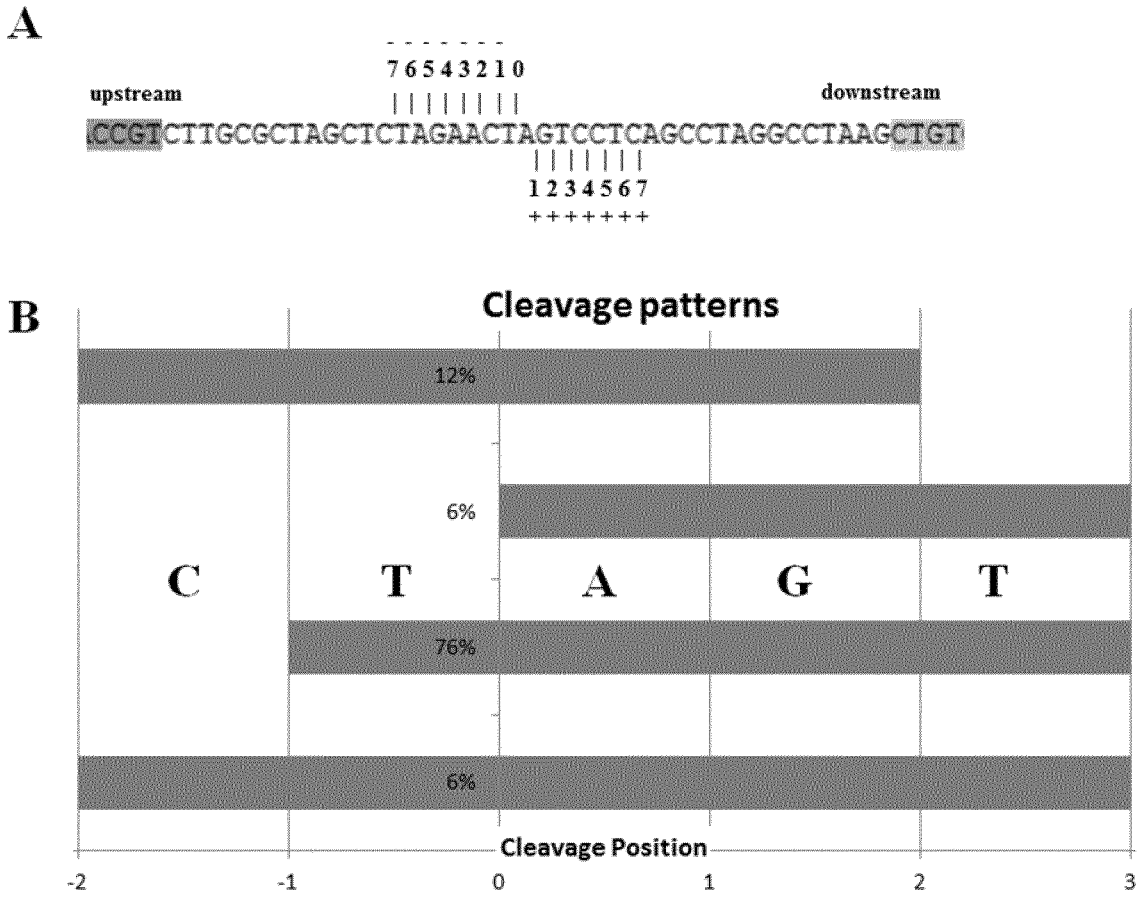


Figure 15


A
7 6 5 4 3 2 1 0
upstream
downstream
CCGICTIGCGCTAGCICTAGAACTAGICCICAGCCTAGGCCTAAGCIGI
1 2 3 4 5 6 7
+ + + + + + +
B
Cleavage patterns
12%
6%
C
T
A
G
T
76%
6%
Cleavage Position
-2
-1
0
1
2
3