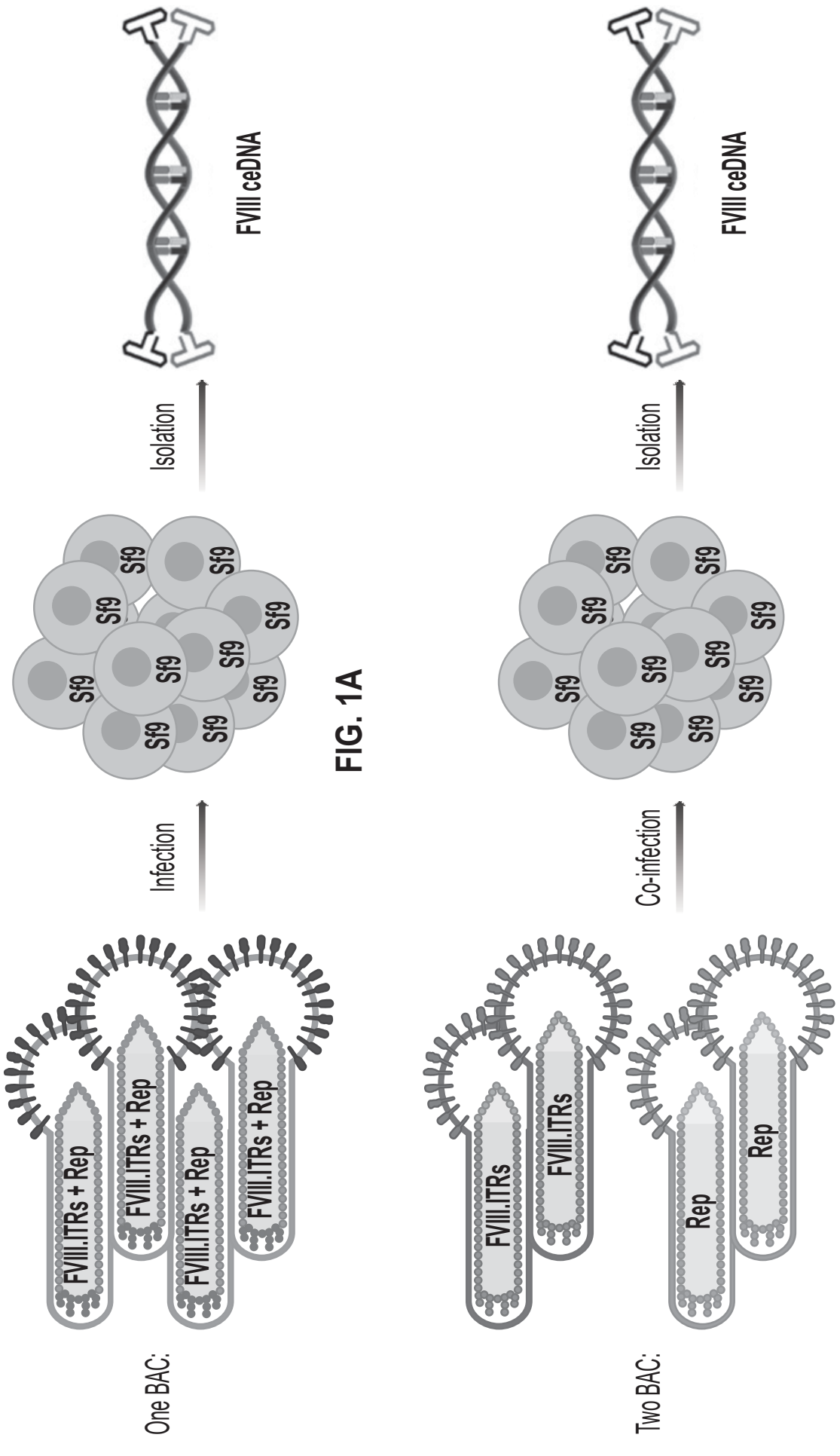

One BAC:
FVIII.ITRs + Rep
FVIII.ITRs + Rep
FVIII.ITRs + Rep
FVIII.ITRs + Rep
Infection
Sf9
Isolation
FVIII ceDNA

FIG. 1A

Two BAC:
FVIII.ITRs
FVIII.ITRs
Rep
Rep
Co-infection
Sf9
Isolation
FVIII ceDNA

FIG. 1B

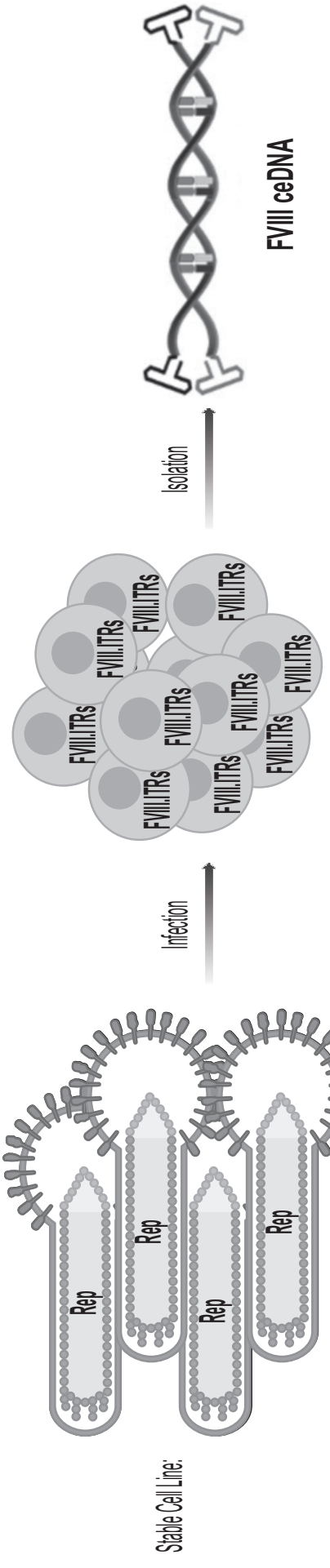

Stable Cell Line:
Rep
Rep
Rep
Rep
Infection
FVIII.ITRs
Isolation
FVIII ceDNA

FIG. 1C

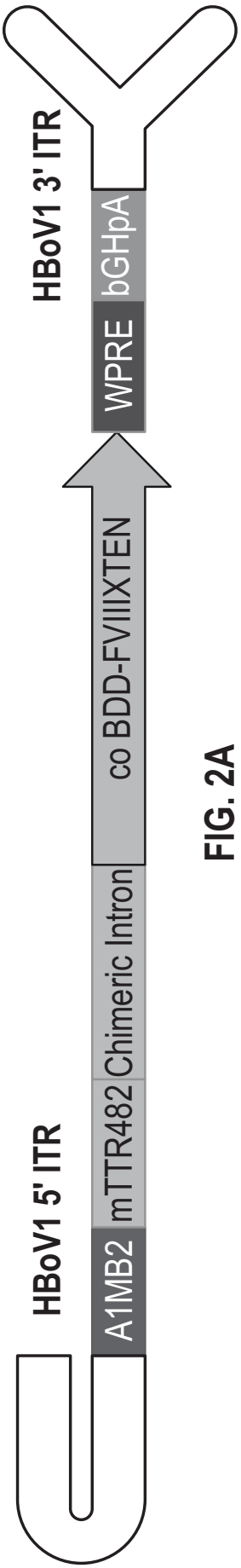

HBoV1 5' ITR
A1MB2
mTTR482
Chimeric Intron
co BDD-FVIIIXTEN
WPRE
bGHpA
HBoV1 3' ITR

FIG. 2A

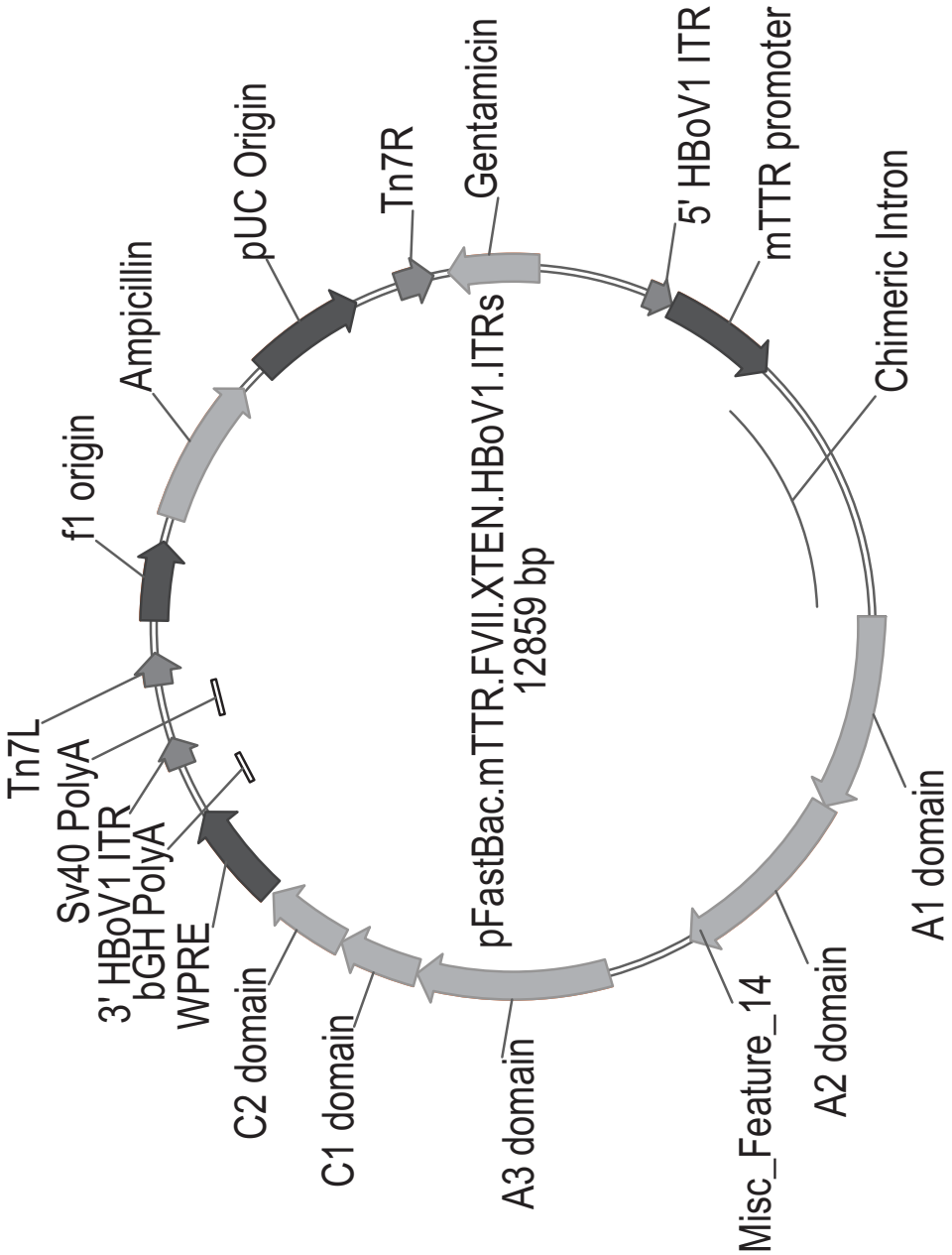

pFastBac.mTTR.FVII.XTEN.HBoV1.ITRs
12859 bp
Tn7L
Sv40 PolyA
3' HBoV1 ITR
bGH PolyA
WPRE
C2 domain
C1 domain
A3 domain
Misc_Feature_14
A2 domain
A1 domain
Chimeric Intron
mTTR promoter
5' HBoV1 ITR
Gentamicin
Tn7R
pUC Origin
Ampicillin
f1 origin

FIG. 2B

FIG. 3A



FIG. 3B

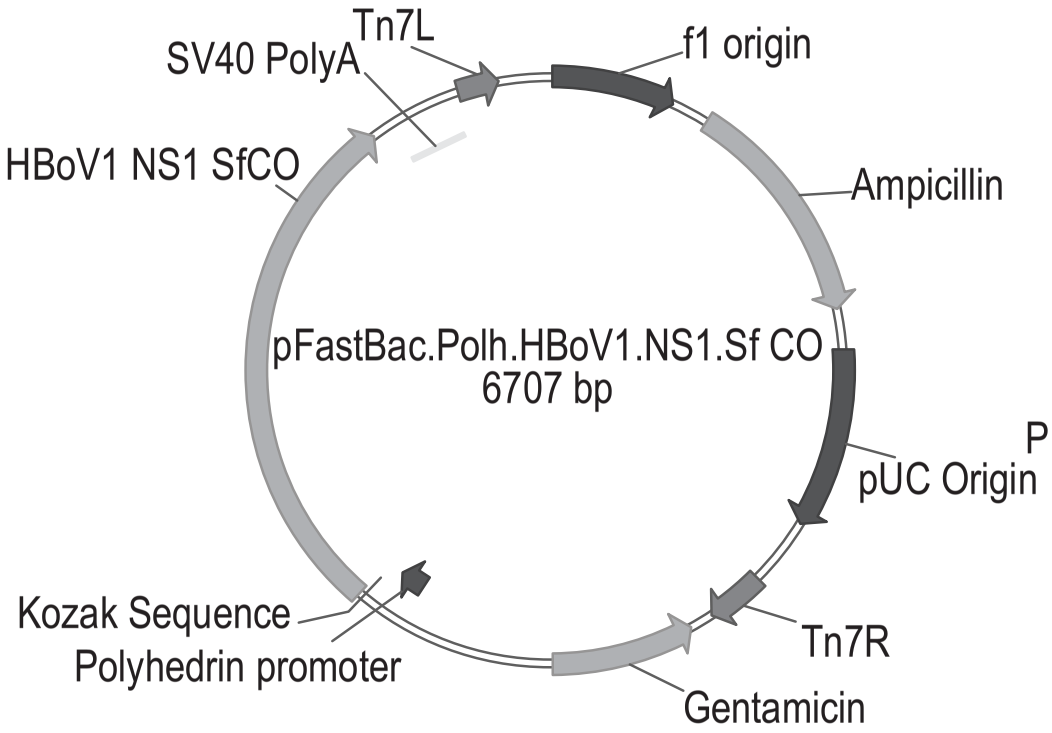


FIG. 3C

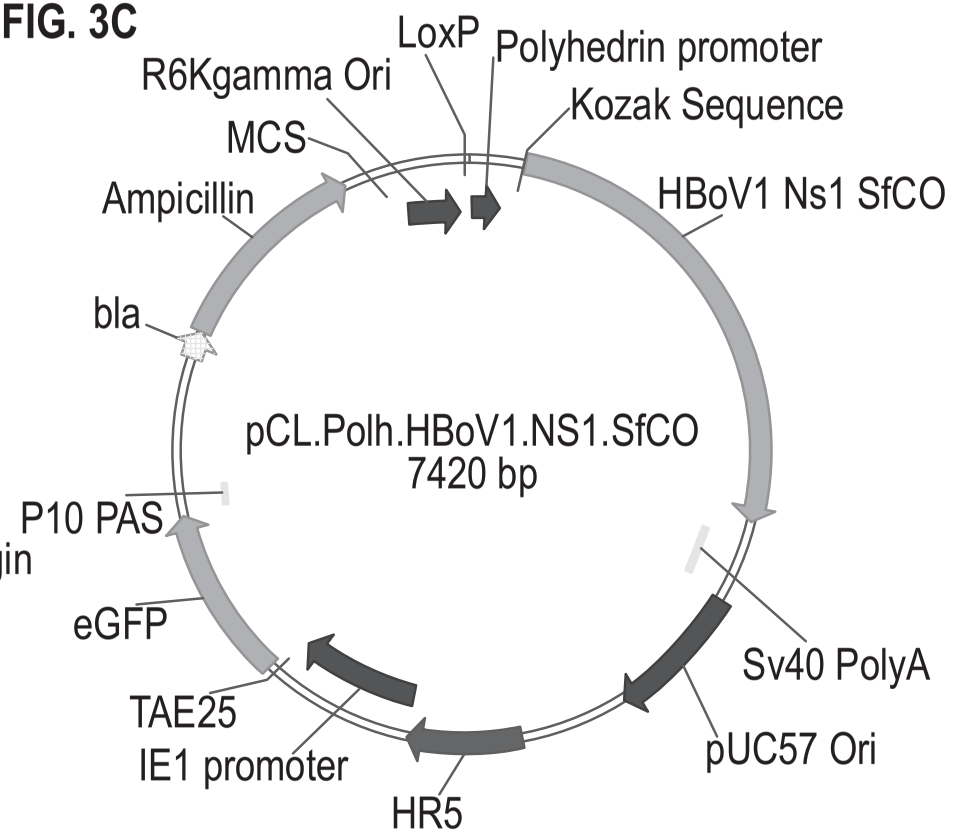


FIG. 4A



FIG. 4B

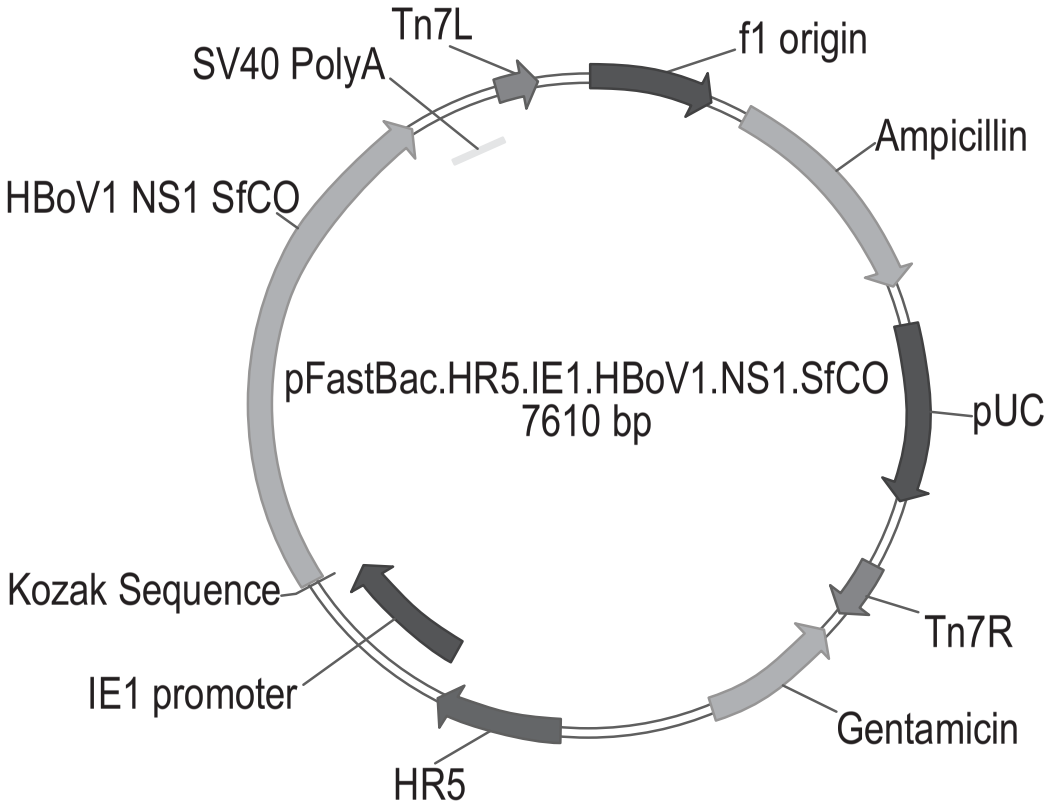


FIG. 4C

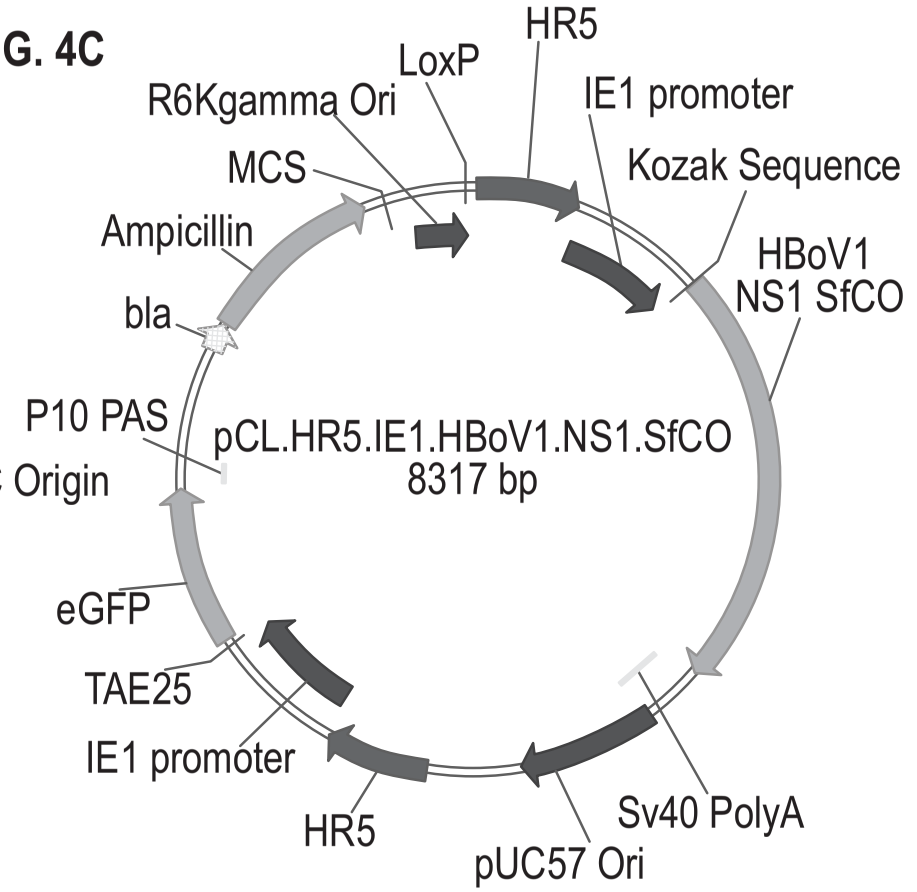


FIG. 5A



FIG. 5B



FIG. 5C

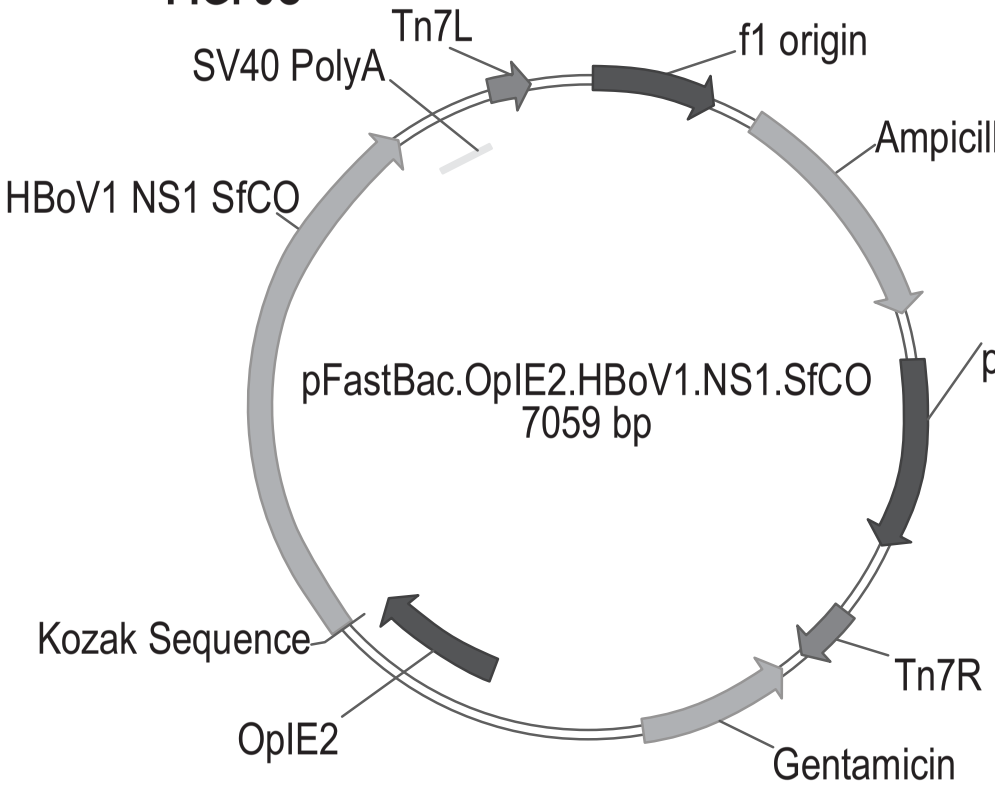
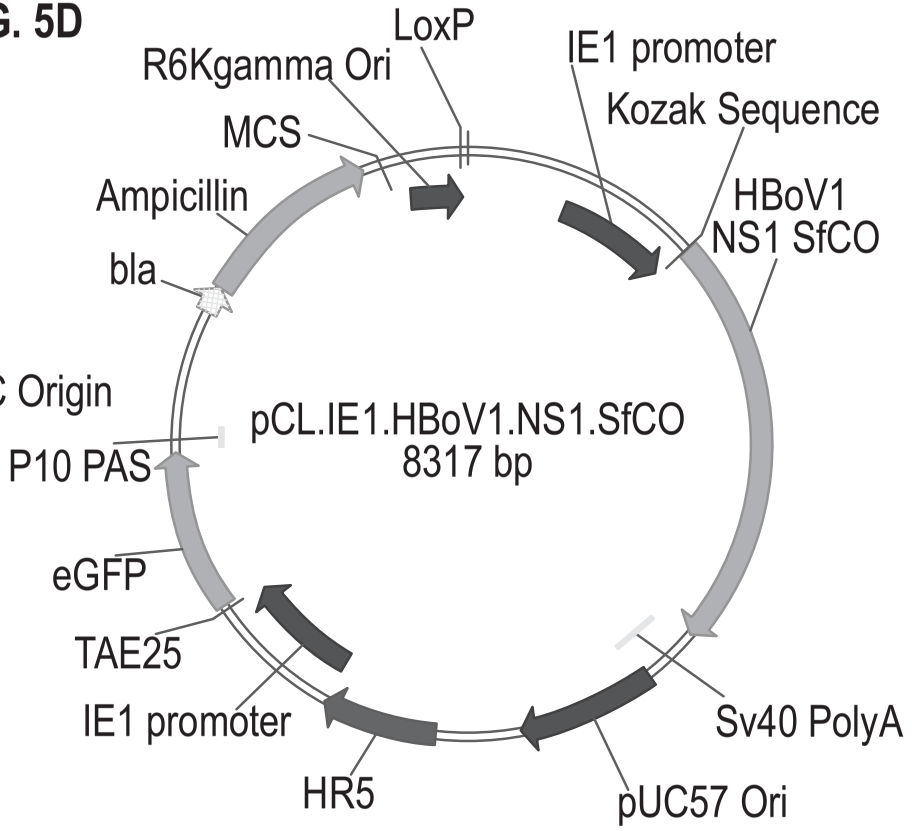


FIG. 5D



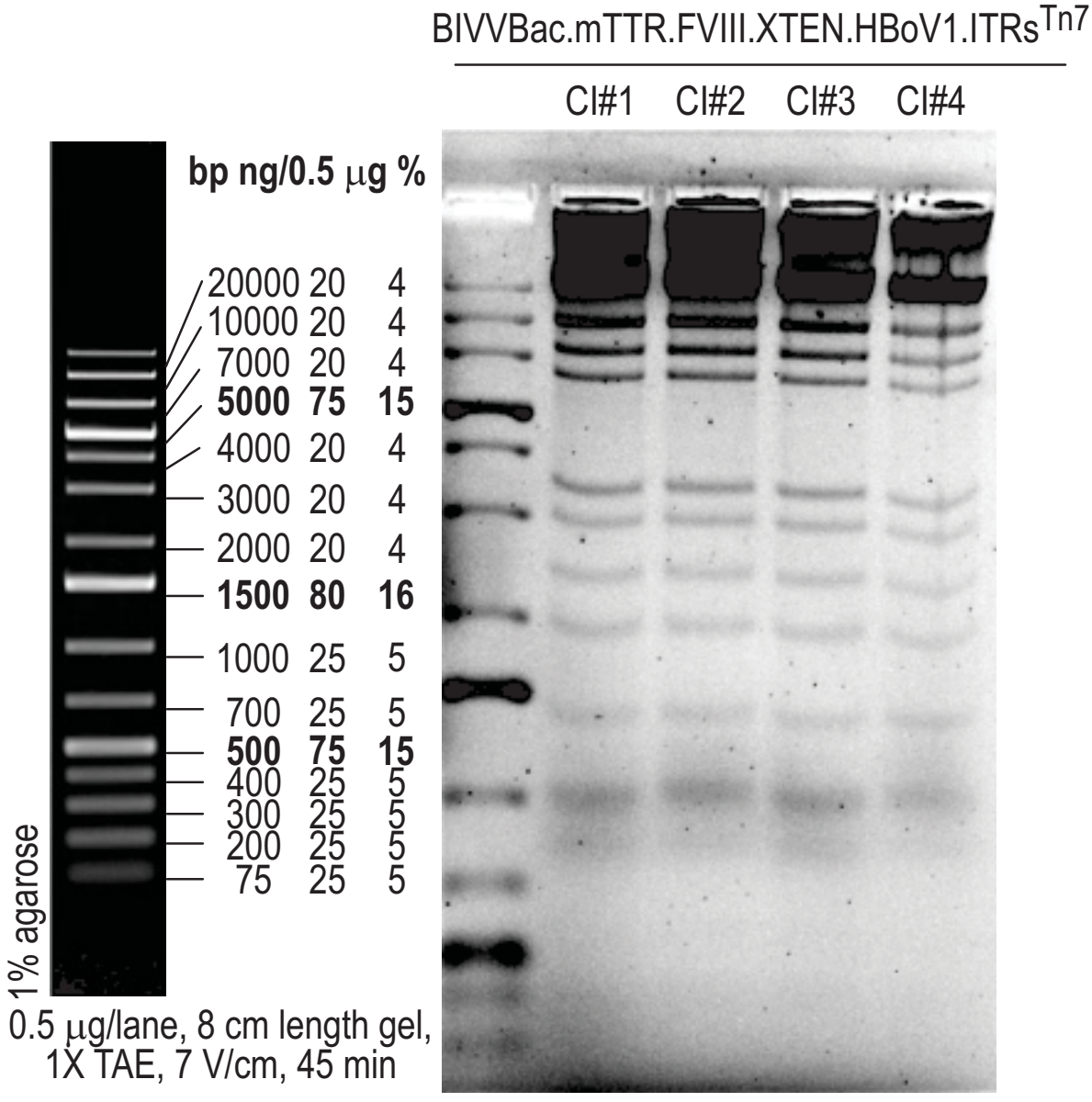

BIVVBac.mTTR.FVIII.XTEN.HBoV1.ITRs Tn7
Cl#1
Cl#2
Cl#3
Cl#4
bp ng/0.5 μg %
20000 20 4
10000 20 4
7000 20 4
5000 75 15
4000 20 4
3000 20 4
2000 20 4
1500 80 16
1000 25 5
700 25 5
500 75 15
400 25 5
300 25 5
200 25 5
75 25 5
1% agarose
0.5 μg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

FIG. 6A

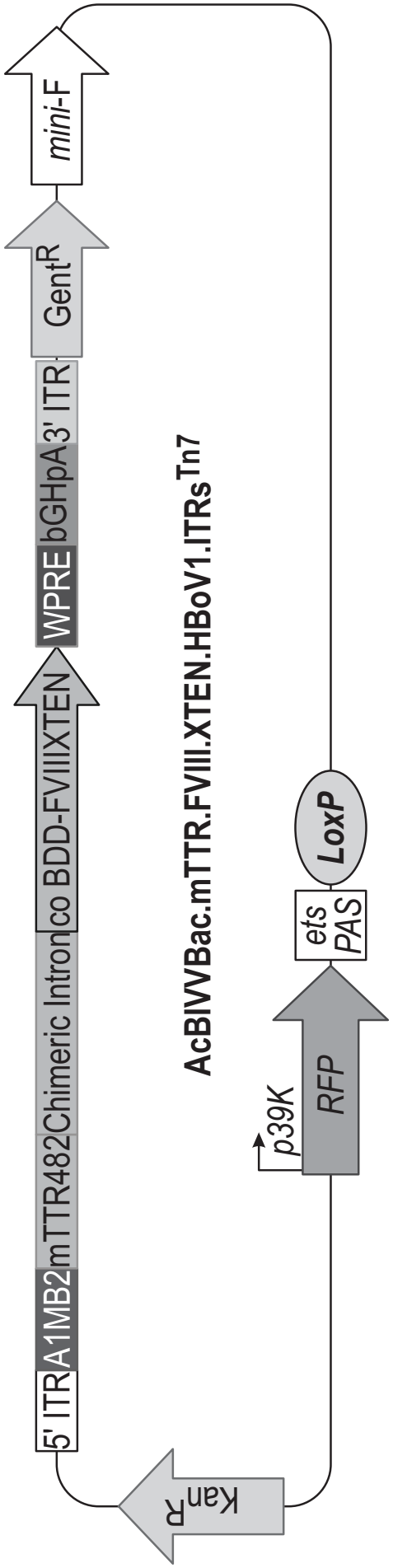

mini-F
GentR
3' ITR
bGHpA
WPRE
co BDD-FVIIIXTEN
Chimeric Intron
mTTR482
MB2
A1
5' ITR
KanR
p39K
RFP
ets
PAS
LoxP
AcBIVVBac.mTTR.FVIII.XTEN.HBoV1.ITRsTn7

FIG. 6B

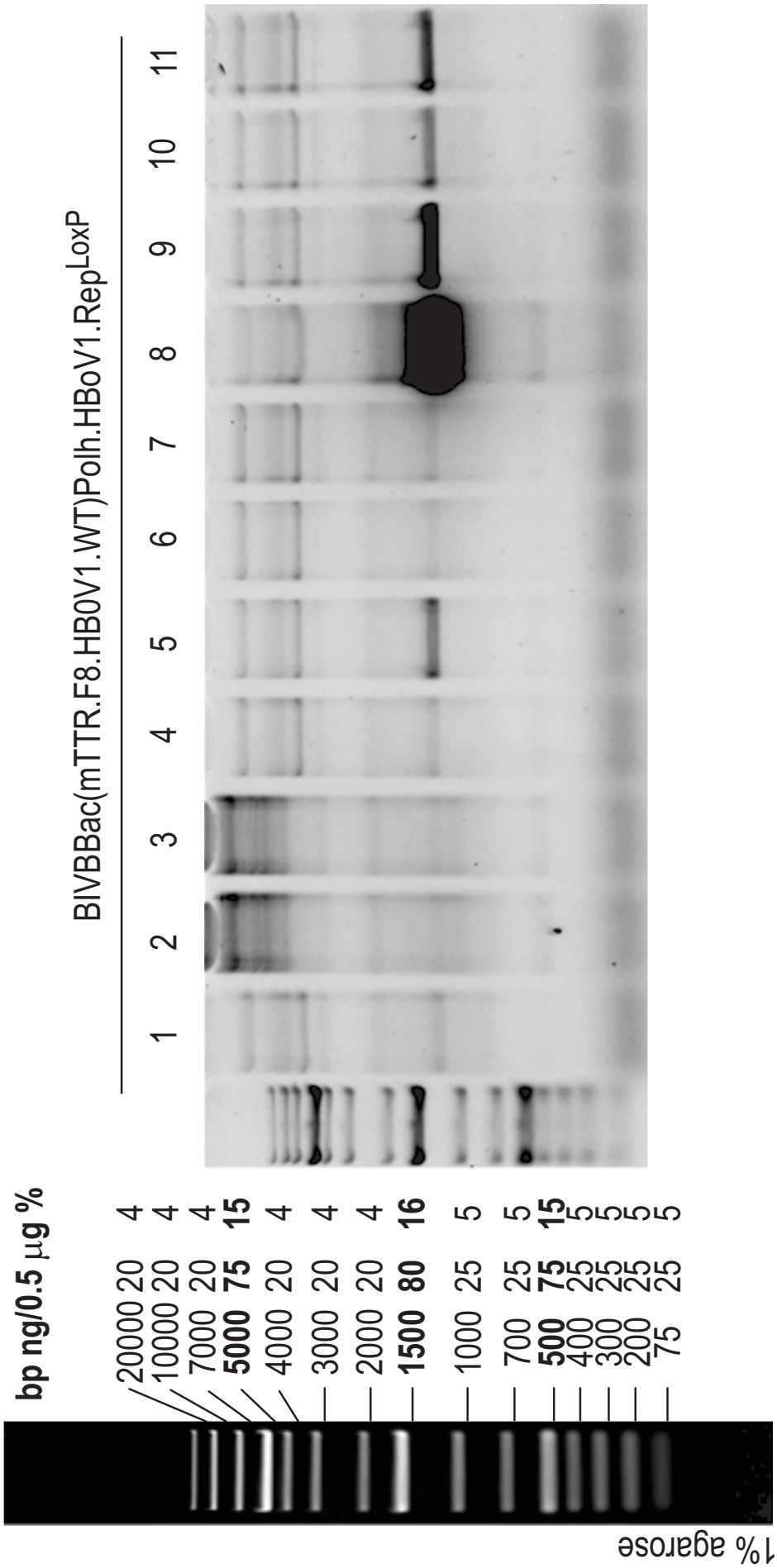

BIVBBac(mTTR.F8.HB0V1.WT)Polh.HB0V1.Rep^{LoxP}
1 2 3 4 5 6 7 8 9 10 11
bp ng/0.5 µg %
20000 20 4
10000 20 4
7000 20 4
5000 75 15
4000 20 4
3000 20 4
2000 20 4
1500 80 16
1000 25 5
700 25 5
500 75 15
400 25 5
300 25 5
200 25 5
75 25 5
1% agarose
0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

FIG. 7A

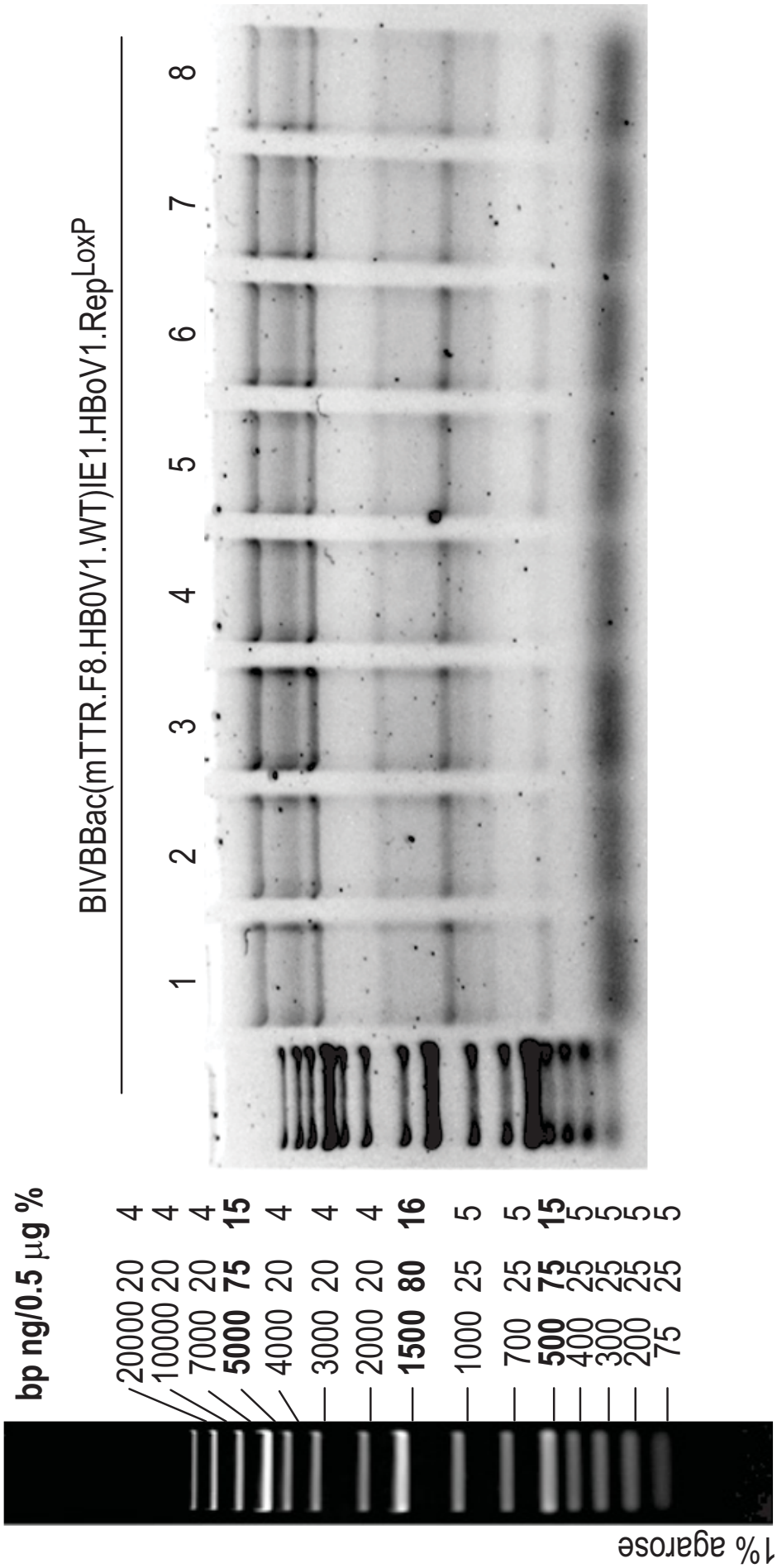

BIVBBac(mTTR.F8.HB0V1.WT)IE1.HBoV1.Rep^{LoxP}
1
2
3
4
5
6
7
8
bp ng/0.5 µg %
20000 20 4
10000 20 4
7000 20 4
5000 75 15
4000 20 4
3000 20 4
2000 20 4
1500 80 16
1000 25 5
700 25 5
500 75 15
400 25 5
300 25 5
200 25 5
75 25 5
1% agarose
0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

FIG. 7B

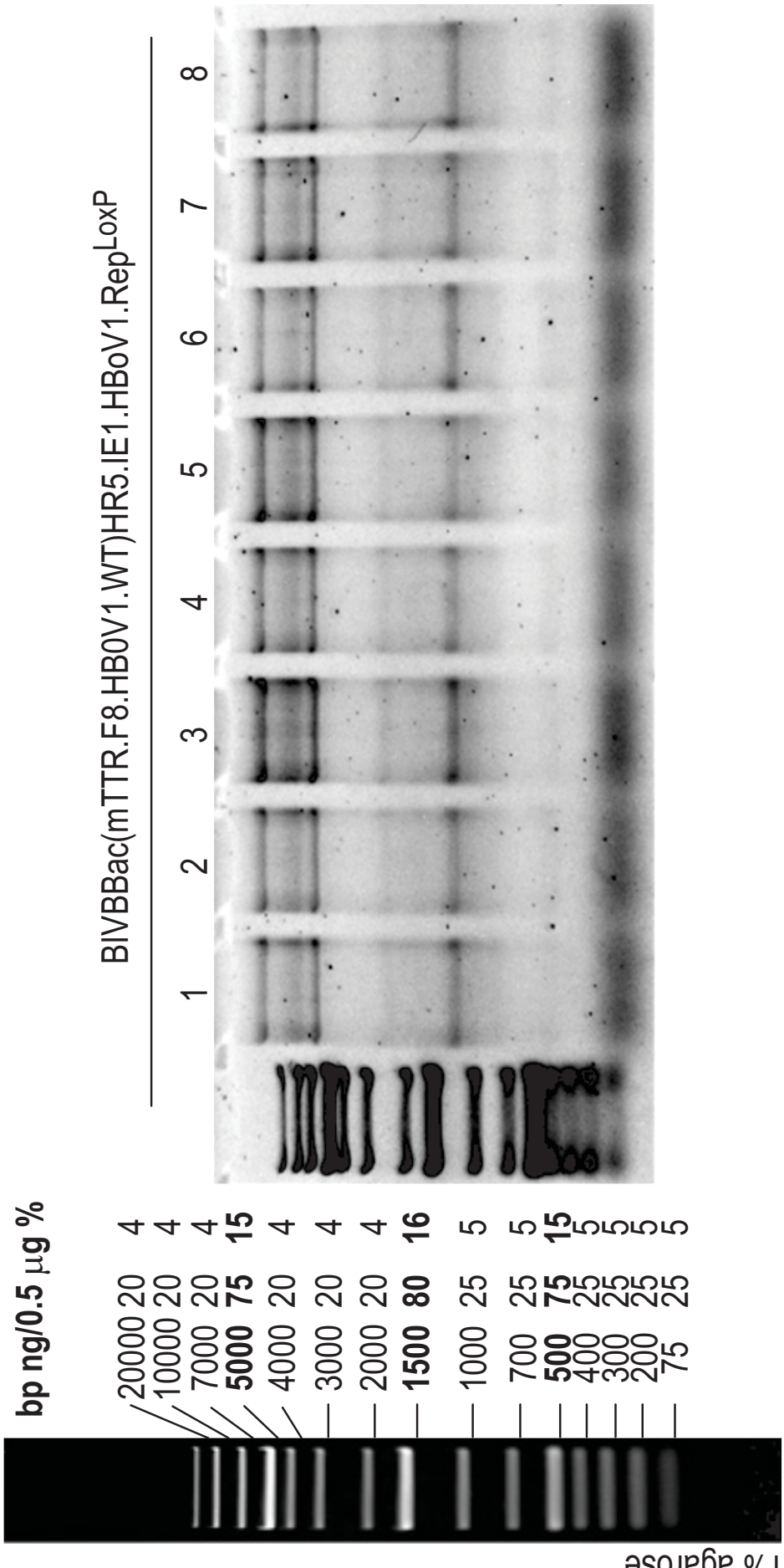

BIVBBac(mTTR.F8.HB0V1.WT)HR5.IE1.HBoV1.RepLoxP
1
2
3
4
5
6
7
8
bp ng/0.5 µg %
20000 20 4
10000 20 4
7000 20 4
5000 75 15
4000 20 4
3000 20 4
2000 20 4
1500 80 16
1000 25 5
700 25 5
500 75 15
400 25 5
300 25 5
200 25 5
75 25 5
1% agarose
0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

FIG. 7C

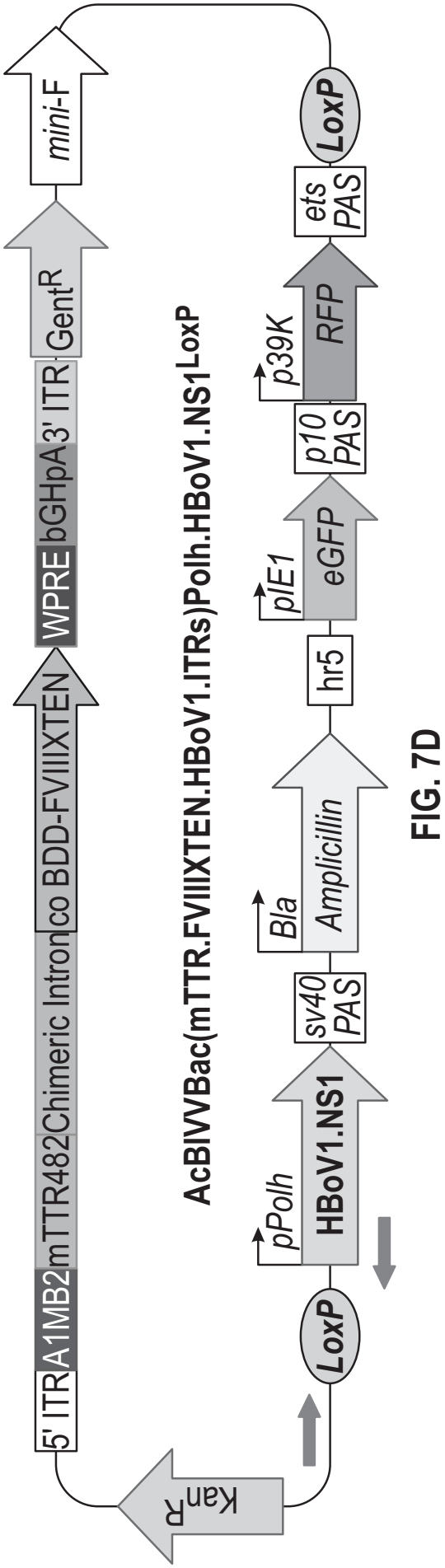

AcBIVVBac(mTTR.FVIIIXTEN.HBoV1.ITRs)Polh.HBoV1.NS1LoxP
mini-F
Gent^R
3' ITR
bGHpA
WPRE
co BDD-FVIIIXTEN
Chimeric Intron
mTTR482
MB2
A1
5' ITR
Kan^R
LoxP
pPolh
HBoV1.NS1
sv40 PAS
Bla
Amplicillin
hr5
pIE1
eGFP
p10 PAS
p39K
RFP
ets PAS
LoxP

FIG. 7D

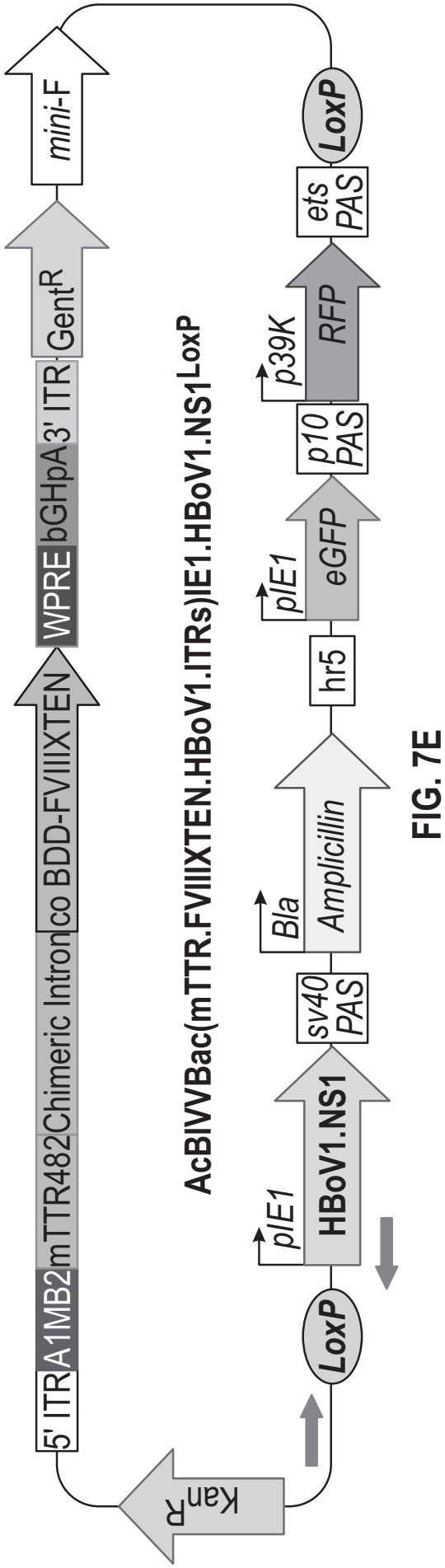

AcBIVVBac(mTTR.FVIIIXTEN.HBoV1.ITRs)IE1.HBoV1.NS1LoxP
mini-F
Gent^R
3' ITR
bGHpA
WPRE
co BDD-FVIIIXTEN
Chimeric Intron
mTTR482
MB2
A1
5' ITR
Kan^R
LoxP
pIE1
HBoV1.NS1
sv40 PAS
Bla
Amplicillin
hr5
pIE1
eGFP
p10 PAS
p39K
RFP
ets PAS
LoxP

FIG. 7E

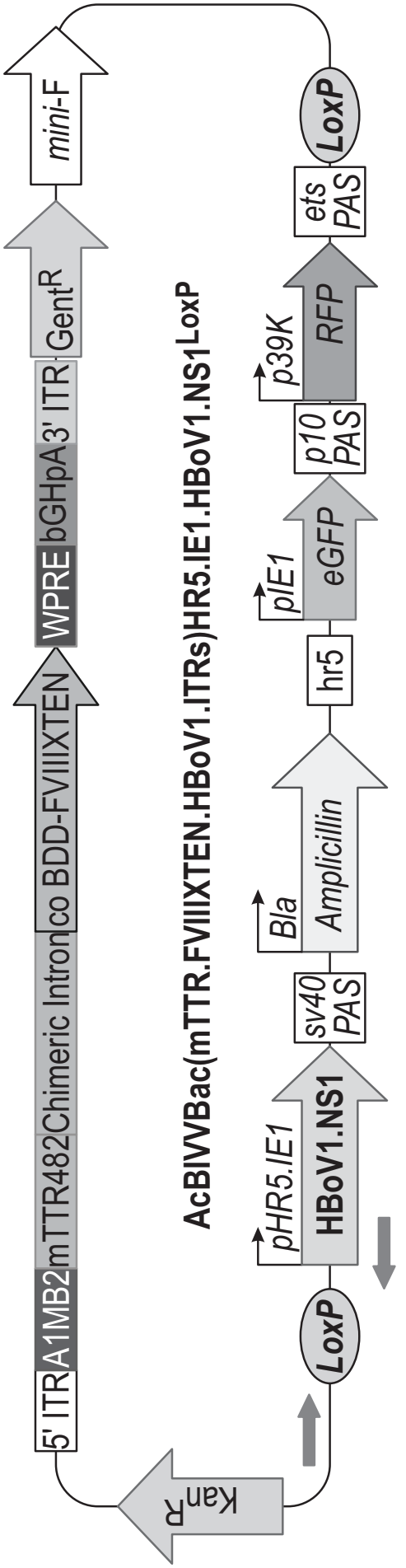

AcBIVVBac(mTTR.FVIIIXTEN.HBoV1.ITRs)HR5.IE1.HBoV1.NS1LoxP
5' ITR
A1MB2
mTTR482
Chimeric Intron
co BDD-FVIIIXTEN
WPRE
bGHpA
3' ITR
Gent^R
mini-F
Kan^R
LoxP
pHR5.IE1
HBoV1.NS1
sv40 PAS
Bla
Amplicillin
hr5
pIE1
eGFP
p10 PAS
p39K
RFP
ets PAS
LoxP

FIG. 7F

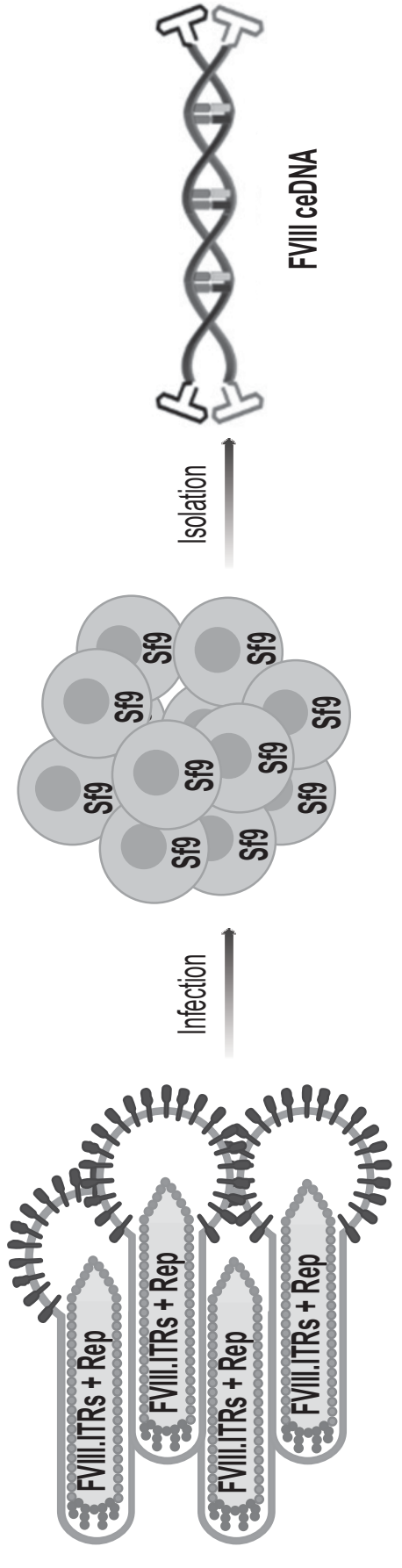

FVIII.ITRs + Rep
FVIII.ITRs + Rep
FVIII.ITRs + Rep
FVIII.ITRs + Rep
Infection
Sf9
Isolation
FVIII ceDNA

FIG. 8A

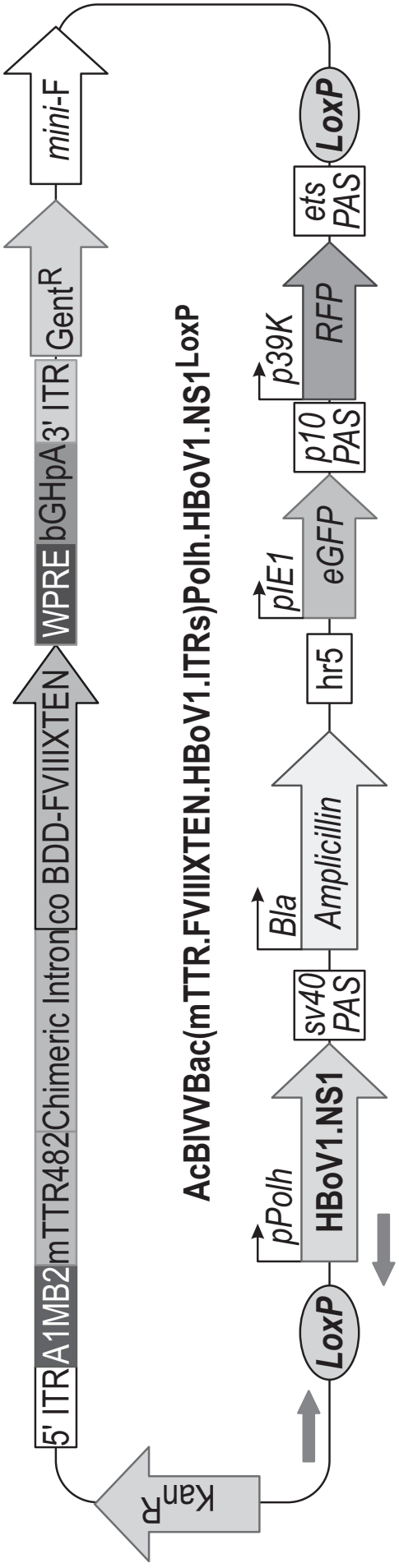

AcBIVVBac(mTTR.FVIIIXTEN.HBoV1.ITRs)Polh.HBoV1.NS1LoxP
mini-F
GentR
3' ITR
bGHpA
WPRE
co BDD-FVIIIXTEN
Chimeric Intron
mTTR482
MB2
A1
5' ITR
KanR
LoxP
pPolh
HBoV1.NS1
sv40 PAS
Bla
Amplicillin
hr5
pIE1
eGFP
p10 PAS
p39K
RFP
ets PAS
LoxP

FIG. 8B

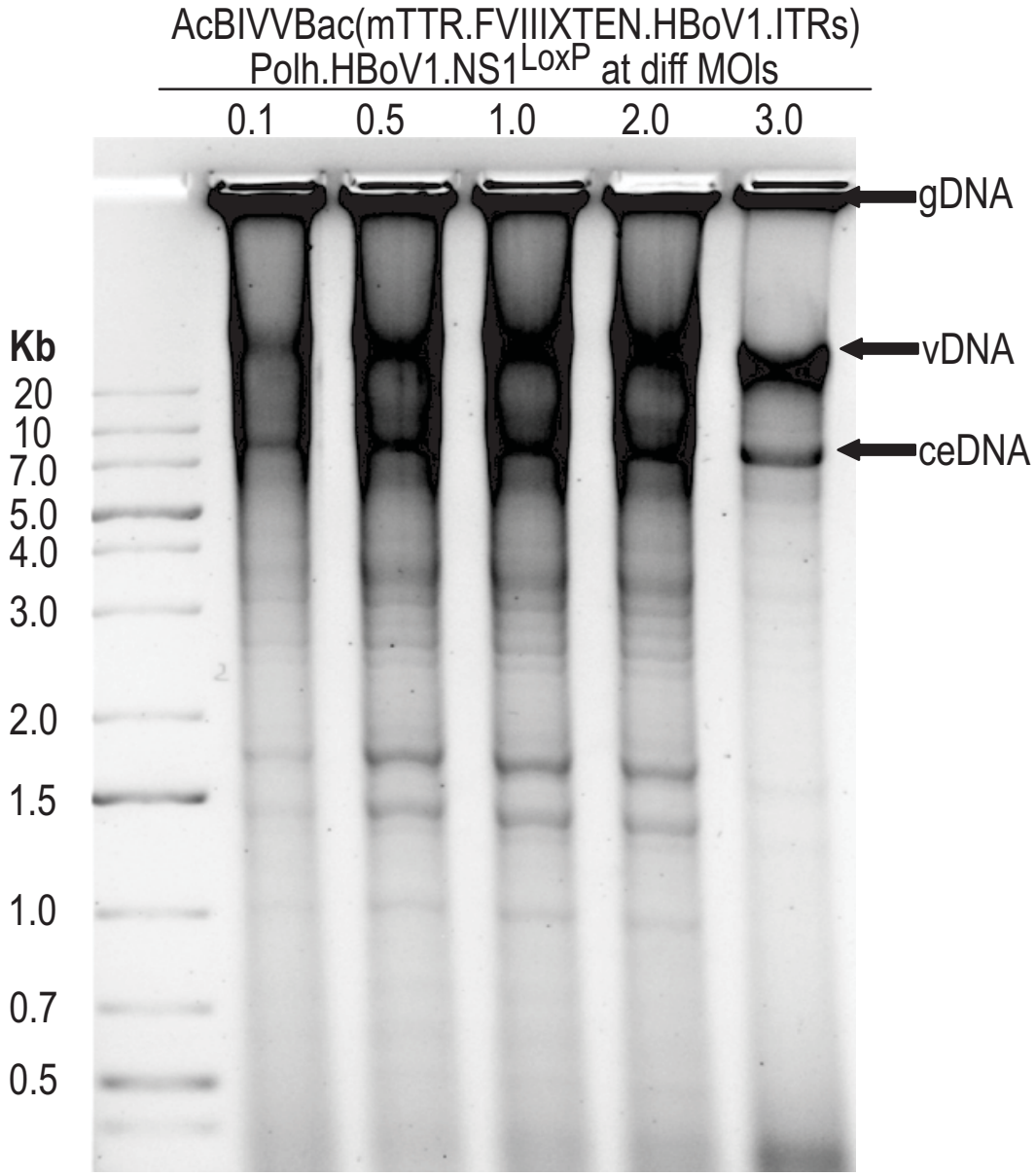

AcBIVVBac(mTTR.FVIIIXTEN.HBoV1.ITRs)
Polh.HBoV1.NS1LoxP at diff MOIs
0.1
0.5
1.0
2.0
3.0
gDNA
vDNA
ceDNA
Kb
20
10
7.0
5.0
4.0
3.0
2.0
1.5
1.0
0.7
0.5

FIG. 8C

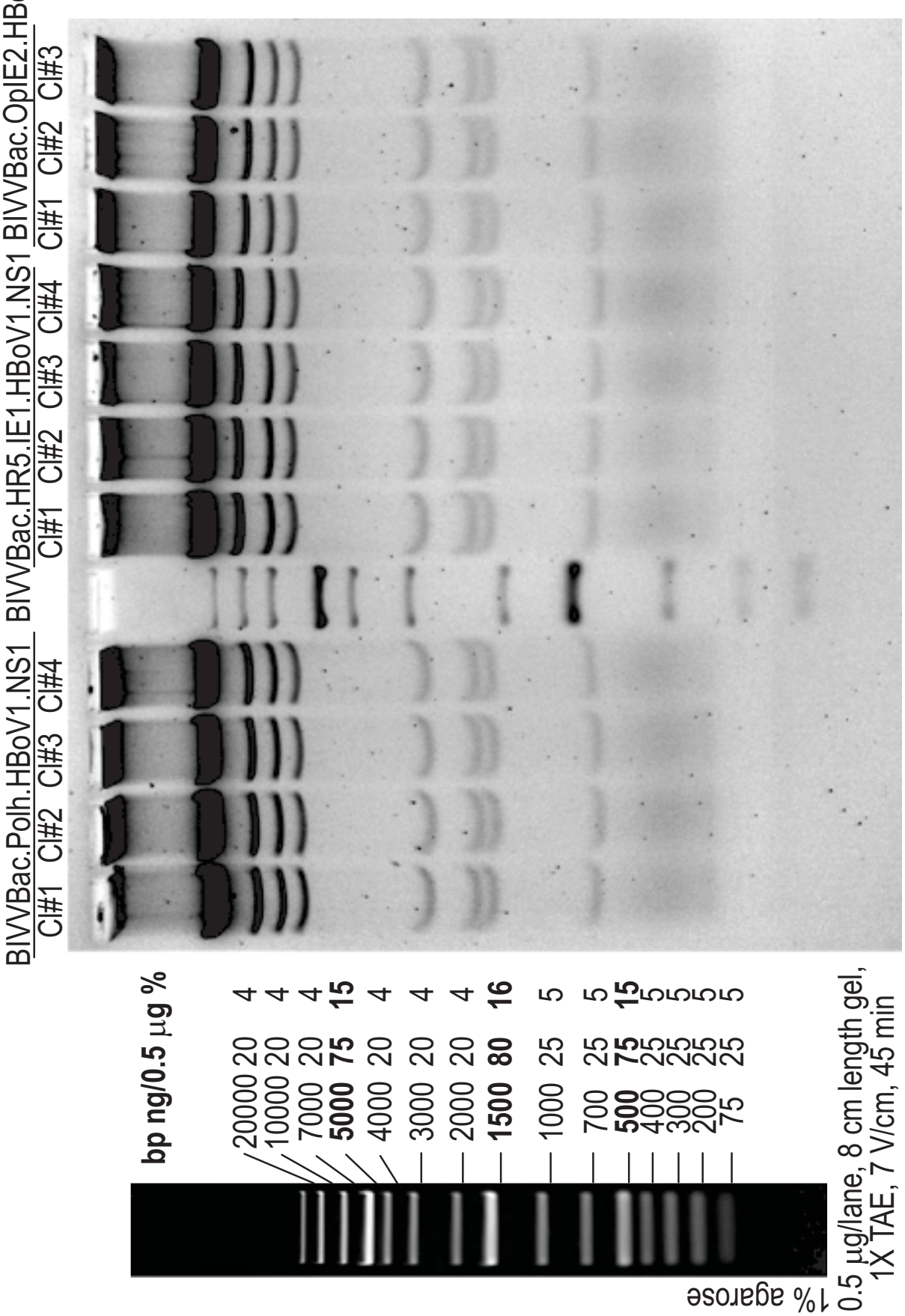

BIVVBac.Polh.HBoV1.NS1
Cl#1 Cl#2 Cl#3 Cl#4
BIVVBac.HR5.IE1.HBoV1.NS1
Cl#1 Cl#2 Cl#3 Cl#4
BIVVBac.OpIE2.HBoV1.NS1
Cl#1 Cl#2 Cl#3
bp ng/0.5 µg %
20000 20 4
10000 20 4
7000 20 4
5000 75 15
4000 20 4
3000 20 4
2000 20 4
1500 80 16
1000 25 5
700 25 5
500 75 15
400 25 5
300 25 5
200 25 5
75 25 5
1% agarose
0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

FIG. 9A

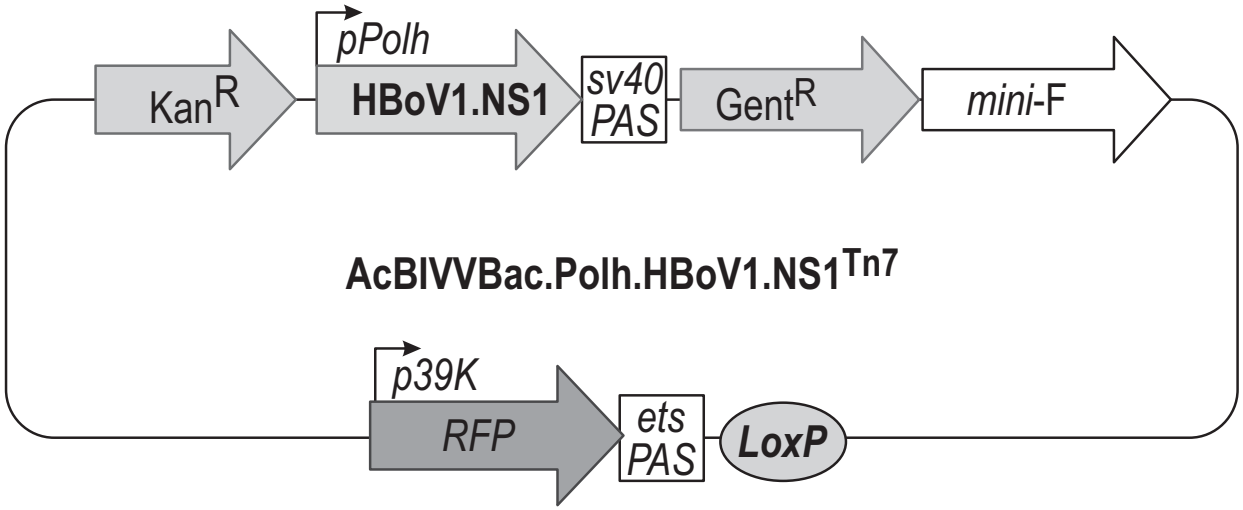


FIG. 9B

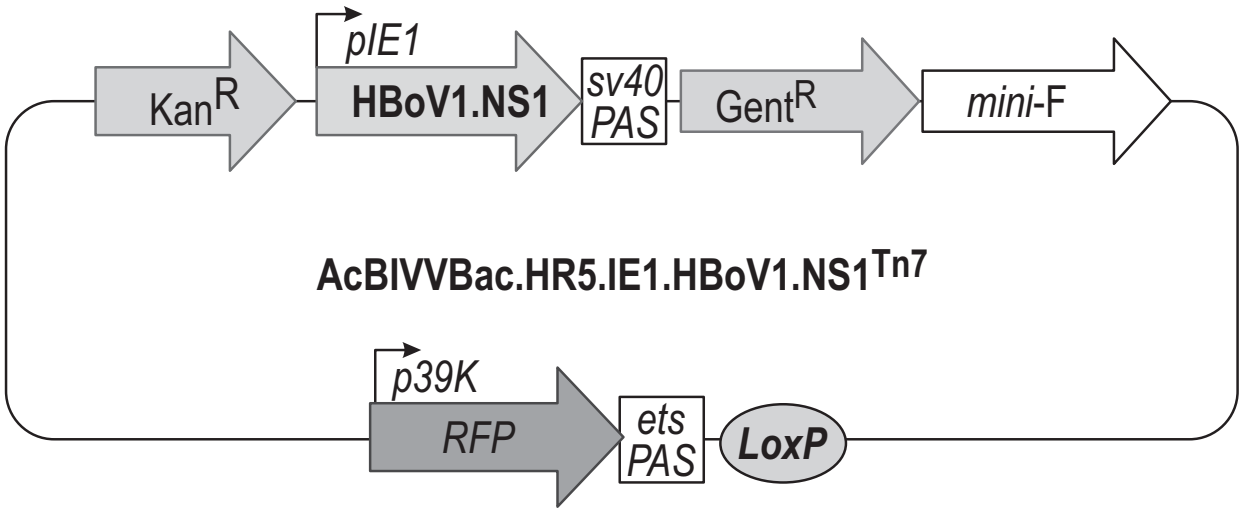


FIG. 9C

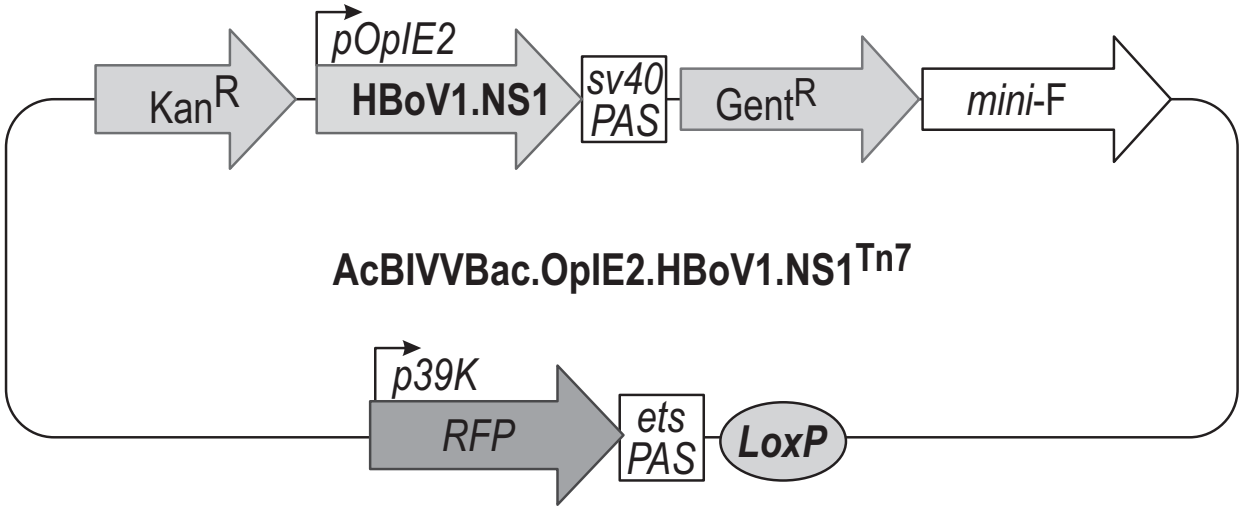


FIG. 9D

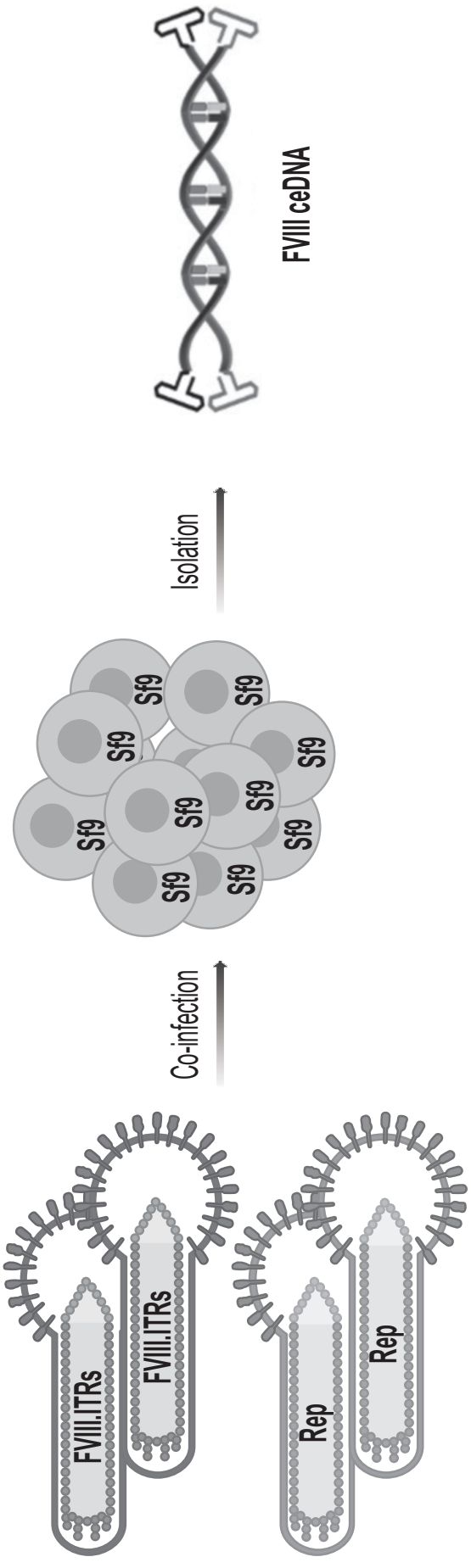

FVIII.ITRs
FVIII.ITRs
Rep
Rep
Co-infection
Sf9
Isolation
FVIII ceDNA

FIG. 10A

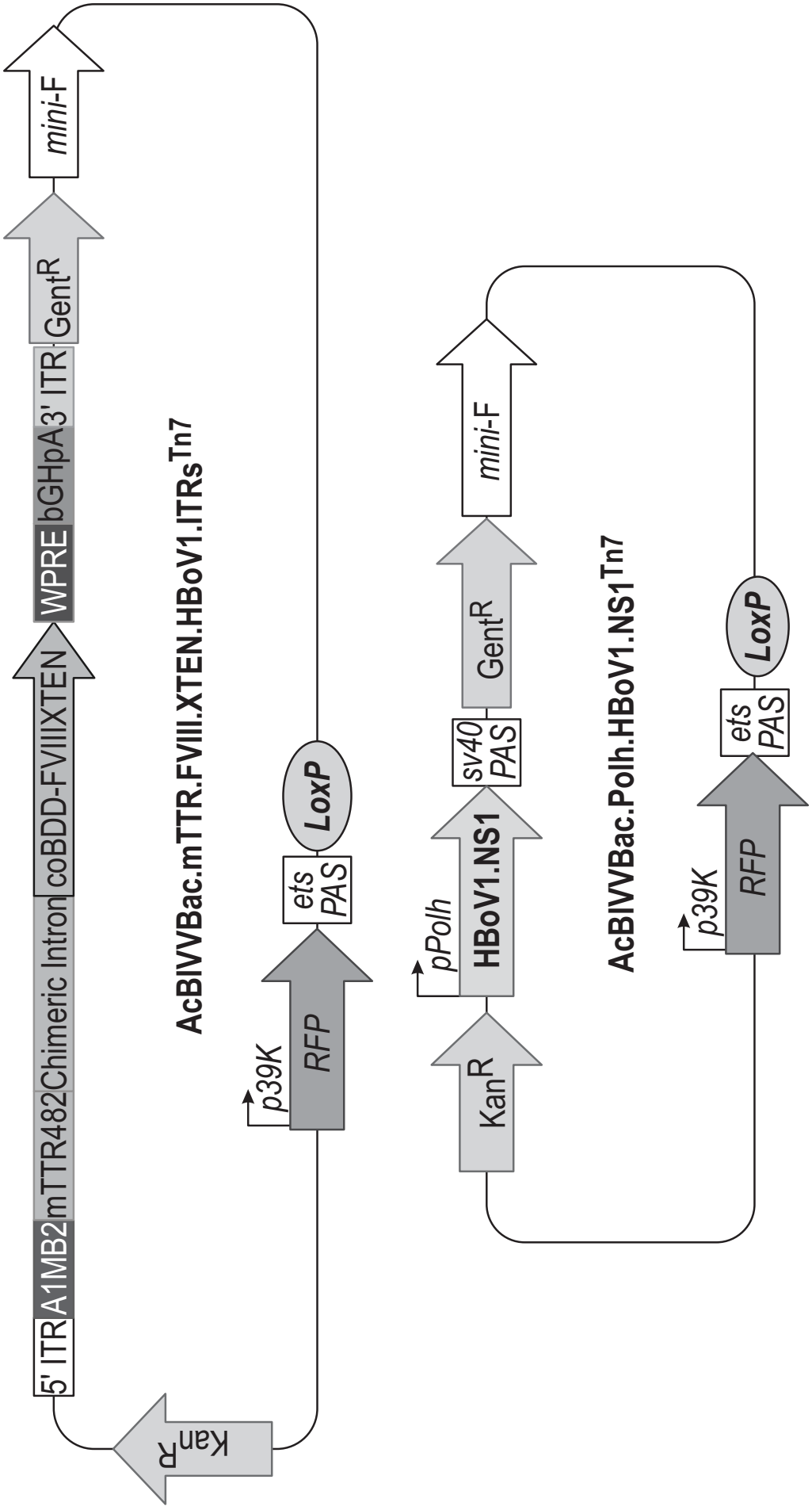

AcBlVVBac.mTTR.FVIII.XTEN.HBoV1.ITRs Tn7
5' ITR
A1MB2
mTTR482
Chimeric Intron
coBDD-FVIIIXTEN
WPRE
bGHpA
3' ITR
Gent R
mini-F
Kan R
p39K
RFP
ets PAS
LoxP
AcBlVVBac.Polh.HBoV1.NS1 Tn7
pPolh
HBoV1.NS1
sv40 PAS
Gent R
mini-F
Kan R
p39K
RFP
ets PAS
LoxP

FIG. 10B

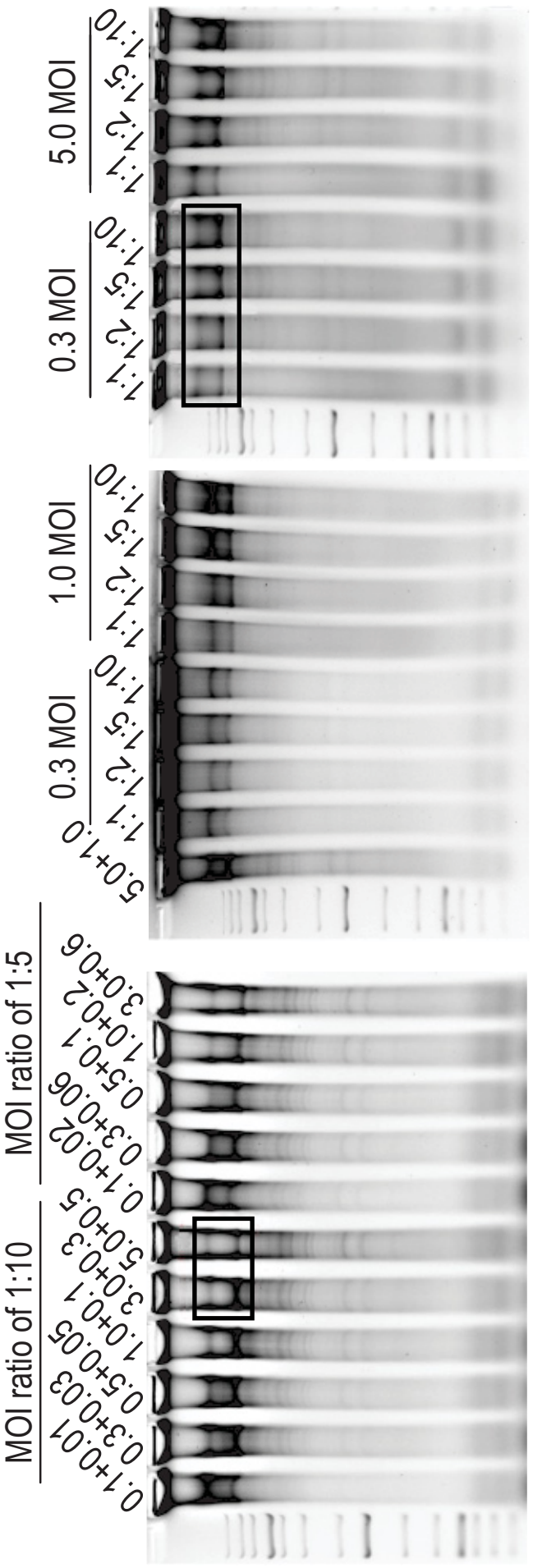

MOI ratio of 1:10
0.1+0.01
0.3+0.03
0.5+0.05
1.0+0.1
3.0+0.3
5.0+0.5
MOI ratio of 1:5
0.1+0.02
0.3+0.06
0.5+0.1
1.0+0.2
3.0+0.6
5.0+1.0
0.3 MOI
1.0 MOI
0.3 MOI
5.0 MOI
1:1
1:2
1:5
1:10

FIG. 10C

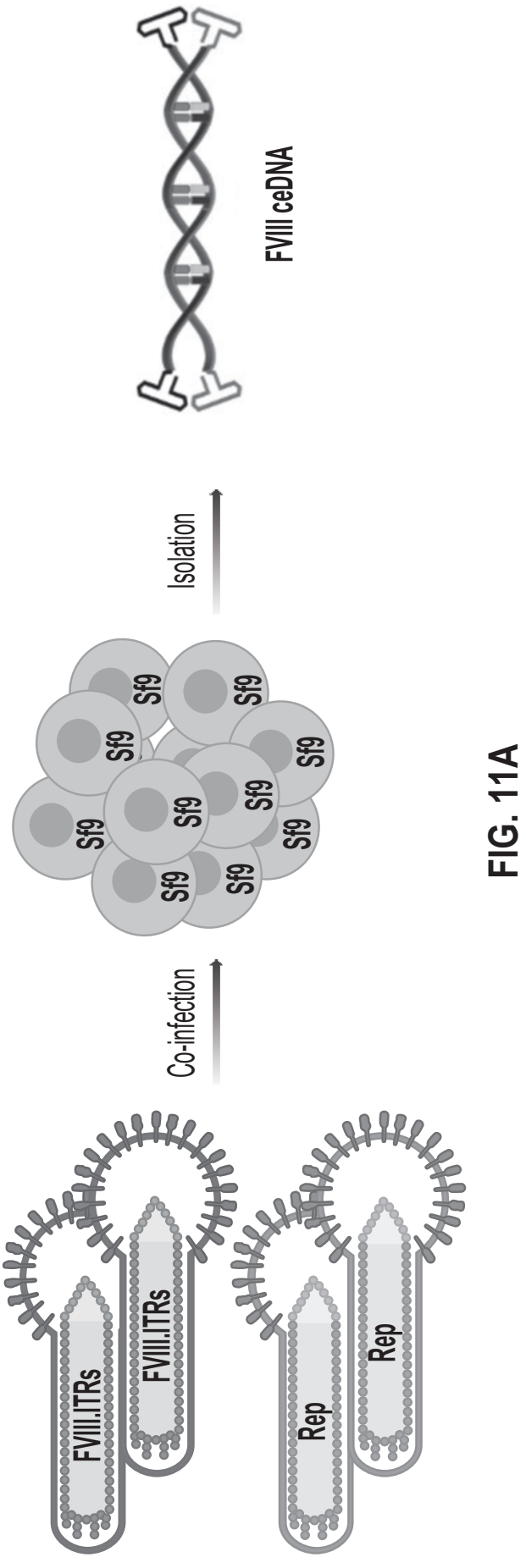

FVIII.ITRs
FVIII.ITRs
Rep
Rep
Co-infection
Sf9
Isolation
FVIII ceDNA

FIG. 11A

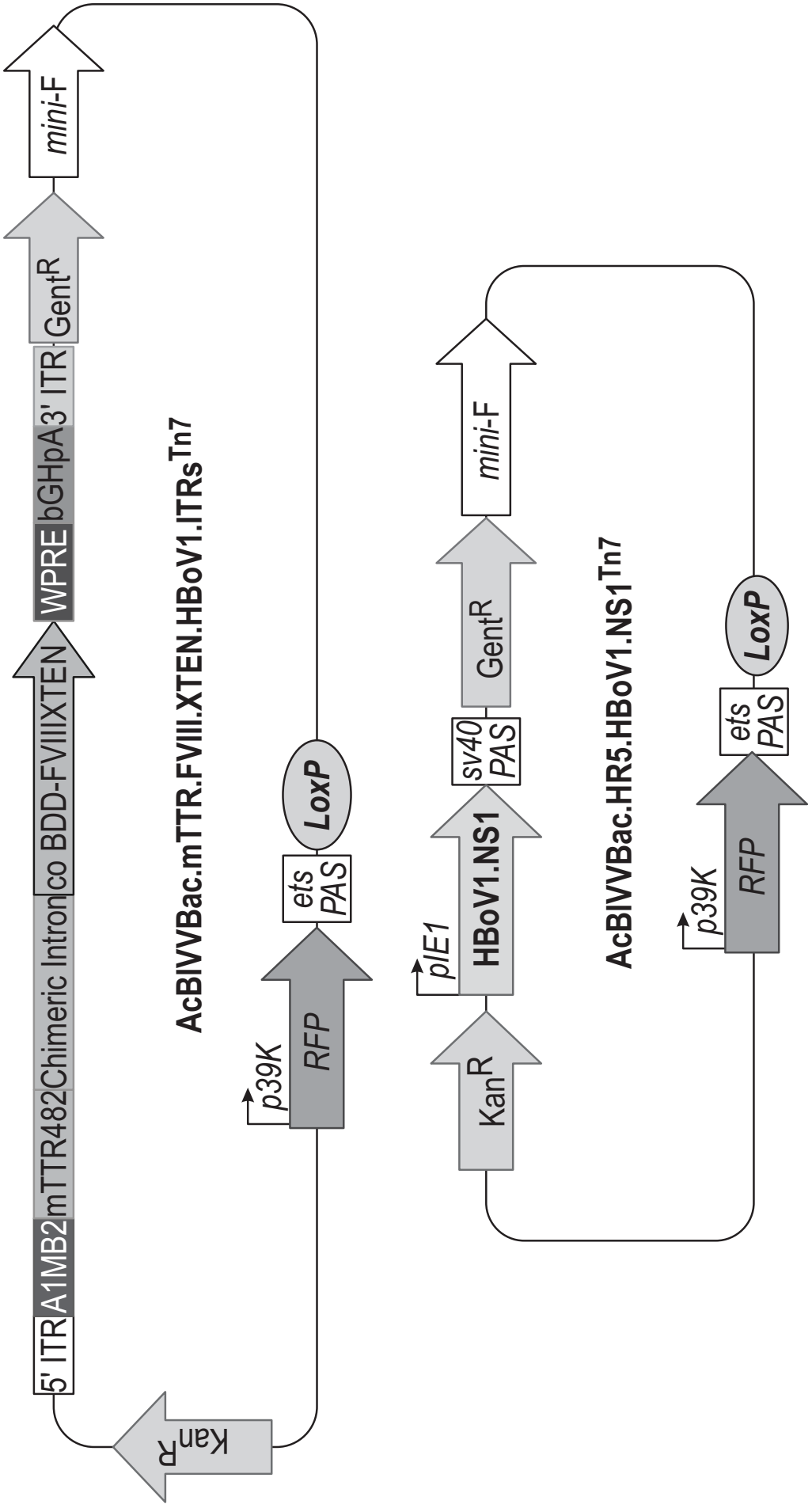

AcBlVVBac.mTTR.FVIII.XTEN.HBoV1.ITRs Tn7
5' ITR
A1MB2
mTTR482
Chimeric Intron
co BDD-FVIIIXTEN
WPRE
bGHpA
3' ITR
Gent R
mini-F
Kan R
p39K
RFP
ets PAS
LoxP
AcBlVVBac.HR5.HBoV1.NS1 Tn7
pIE1
HBoV1.NS1
sv40 PAS
Gent R
mini-F
Kan R
p39K
RFP
ets PAS
LoxP

FIG. 11B

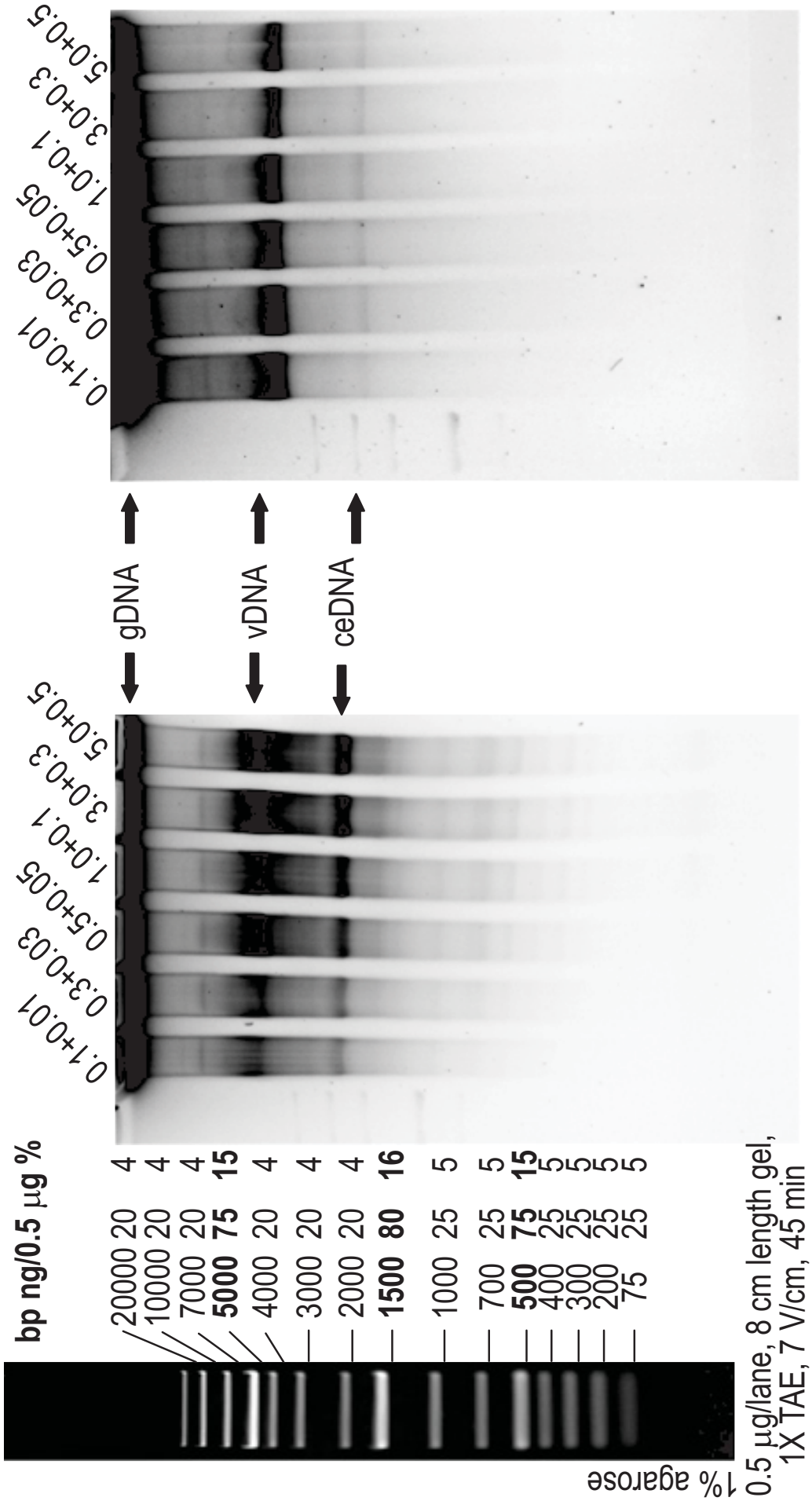

mTTR.FVIII.HBoV1 Cl#5 +
HR5.IE1.NS1 Cl#2 BEVs at diff MOIs
0.1+0.01
0.3+0.03
0.5+0.05
1.0+0.1
3.0+0.3
5.0+0.5
gDNA
vDNA
ceDNA
mTTR.FVIII.HBoV1 Cl#5 +
Polh.NS1 Cl#2 BEVs at diff MOIs
0.1+0.01
0.3+0.03
0.5+0.05
1.0+0.1
3.0+0.3
5.0+0.5
bp ng/0.5 µg %
20000 20 4
10000 20 4
7000 20 4
5000 75 15
4000 20 4
3000 20 4
2000 20 4
1500 80 16
1000 25 5
700 25 5
500 75 15
400 25 5
300 25 5
200 25 5
75 25 5
1% agarose
0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

FIG. 11C

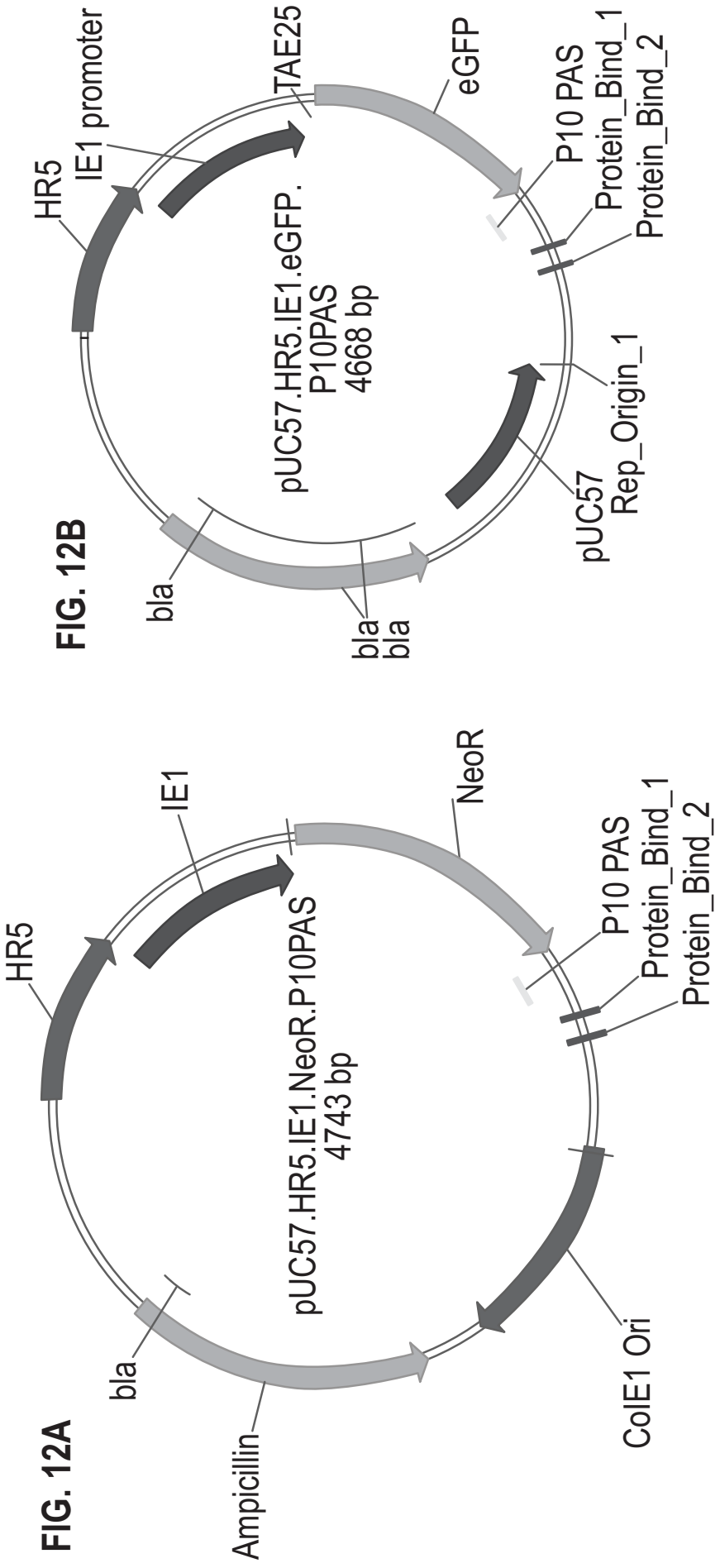


FIG. 12A

FIG. 12B

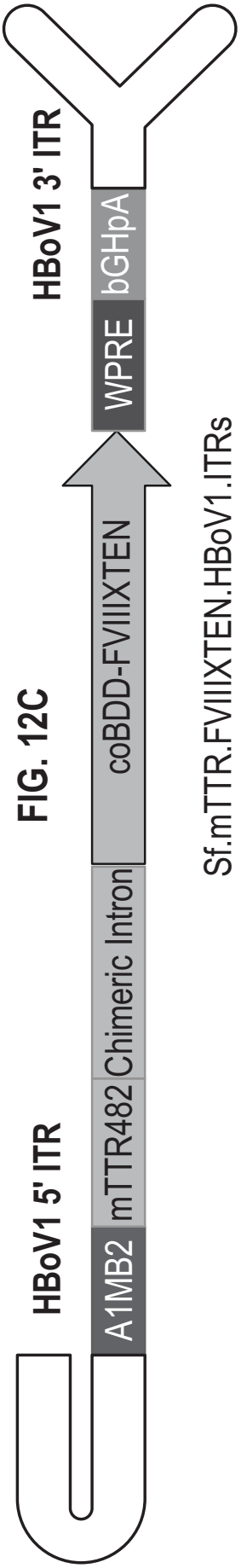


FIG. 12C

Sf.mTTR.FVIIIXTEN.HBoV1.ITRs

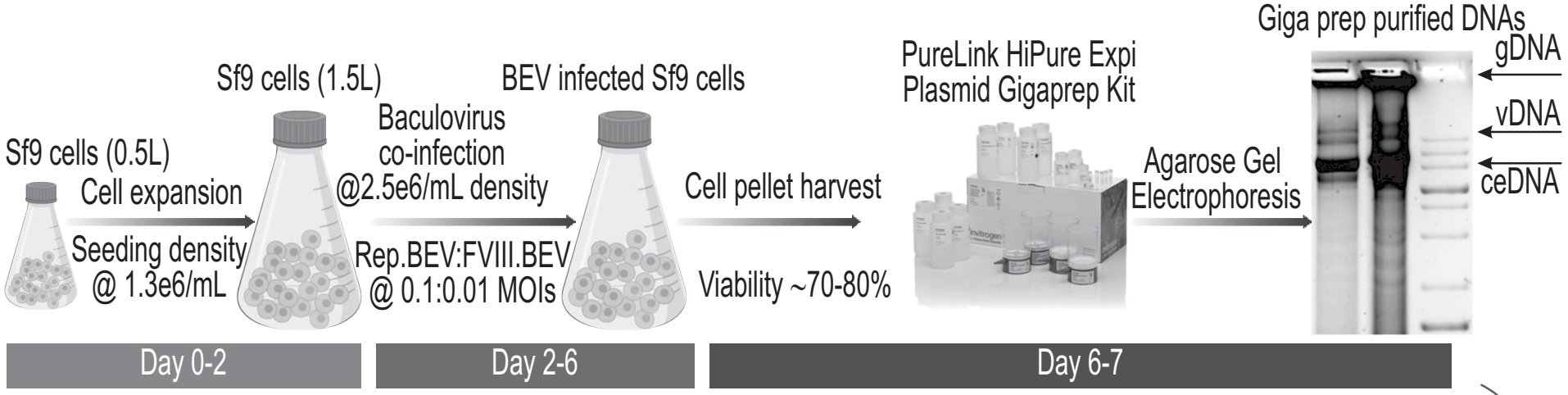


FIG. 13A

FIG. 13B

FIG. 13C

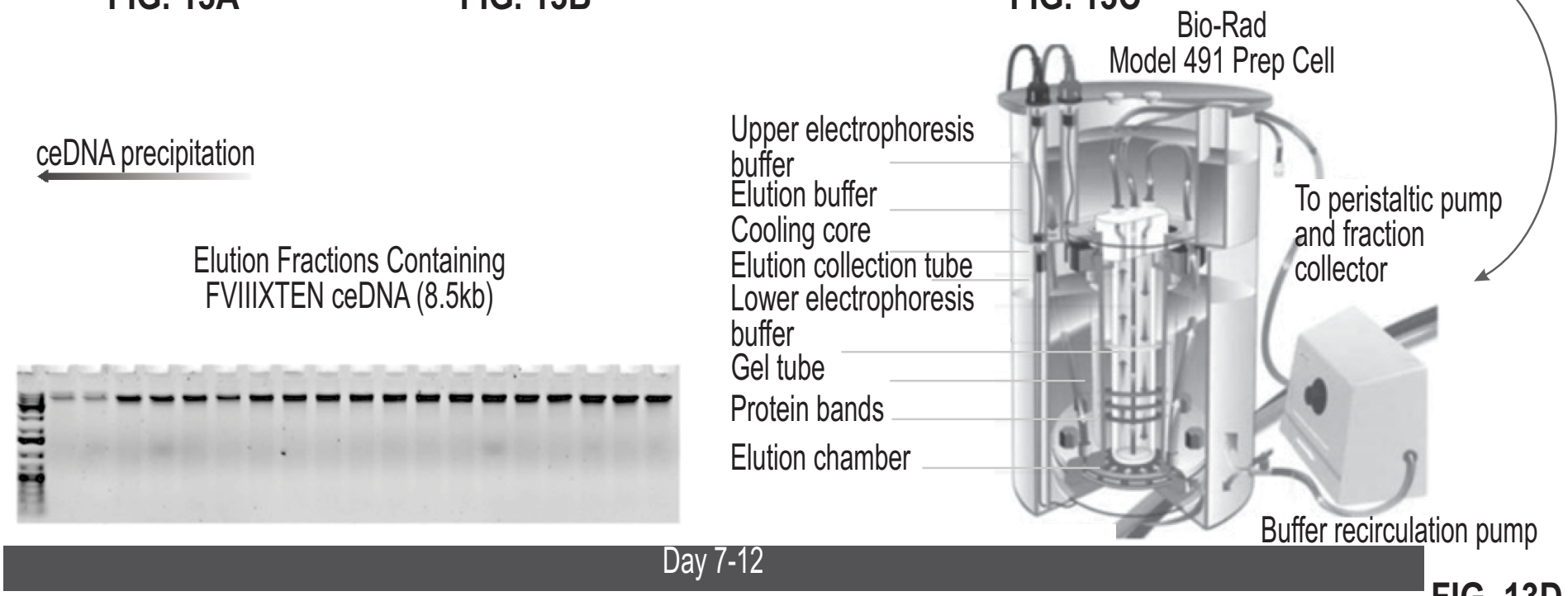


FIG. 13D

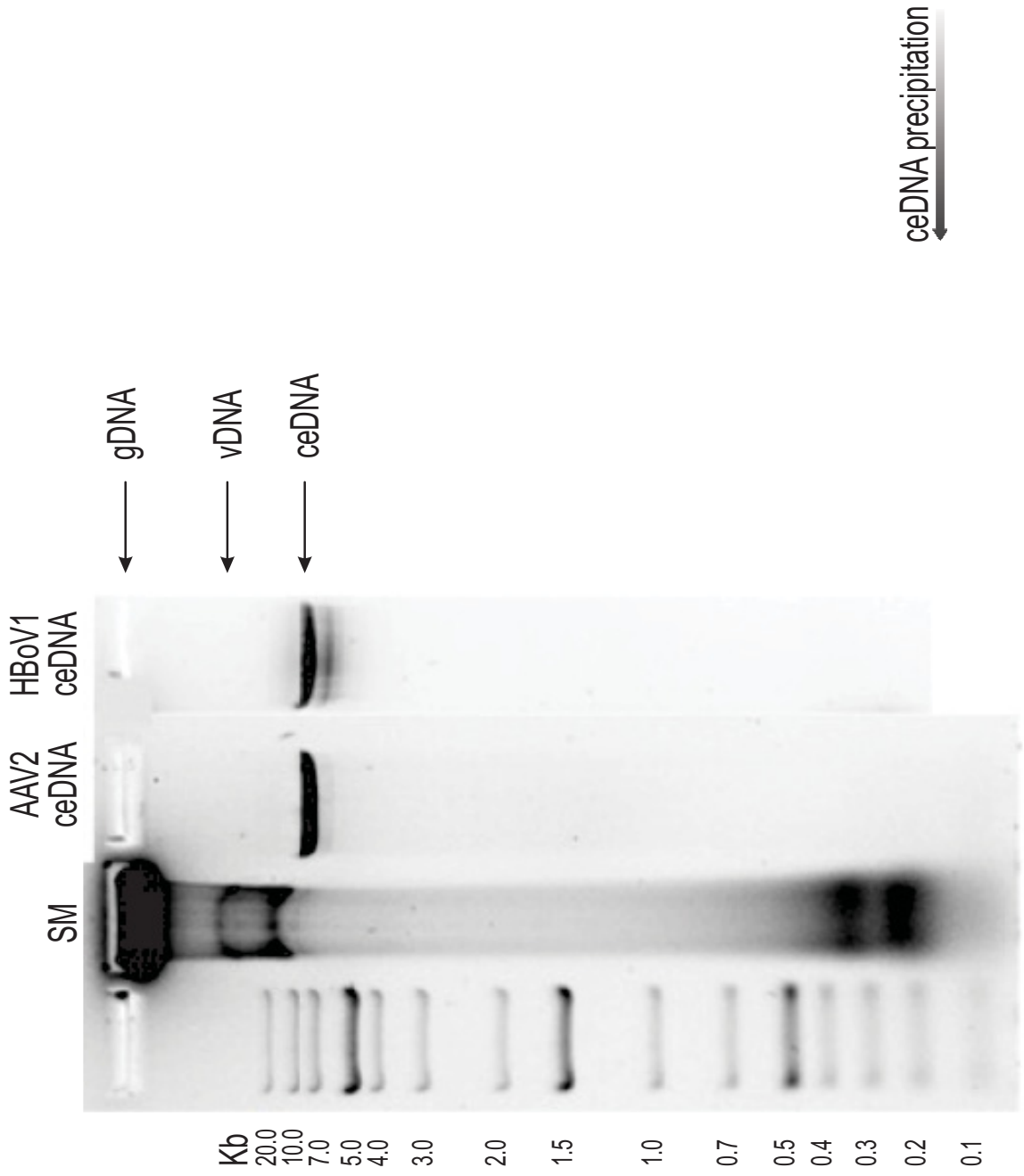

ceDNA precipitation
gDNA
vDNA
ceDNA
HBoV1 ceDNA
AAV2 ceDNA
SM
Kb
20.0
10.0
7.0
5.0
4.0
3.0
2.0
1.5
1.0
0.7
0.5
0.4
0.3
0.2
0.1

FIG. 13E

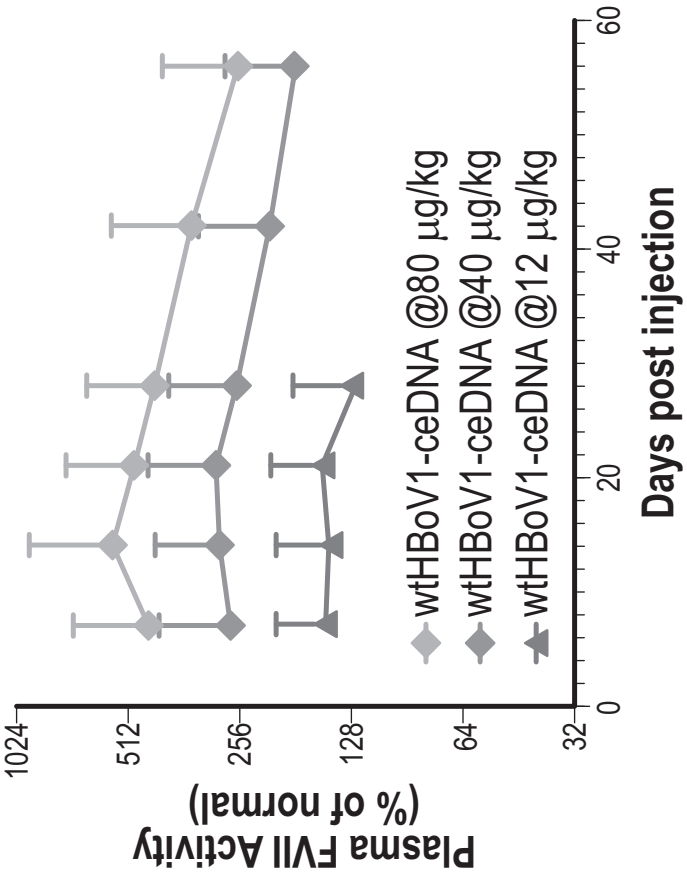


FIG. 14B

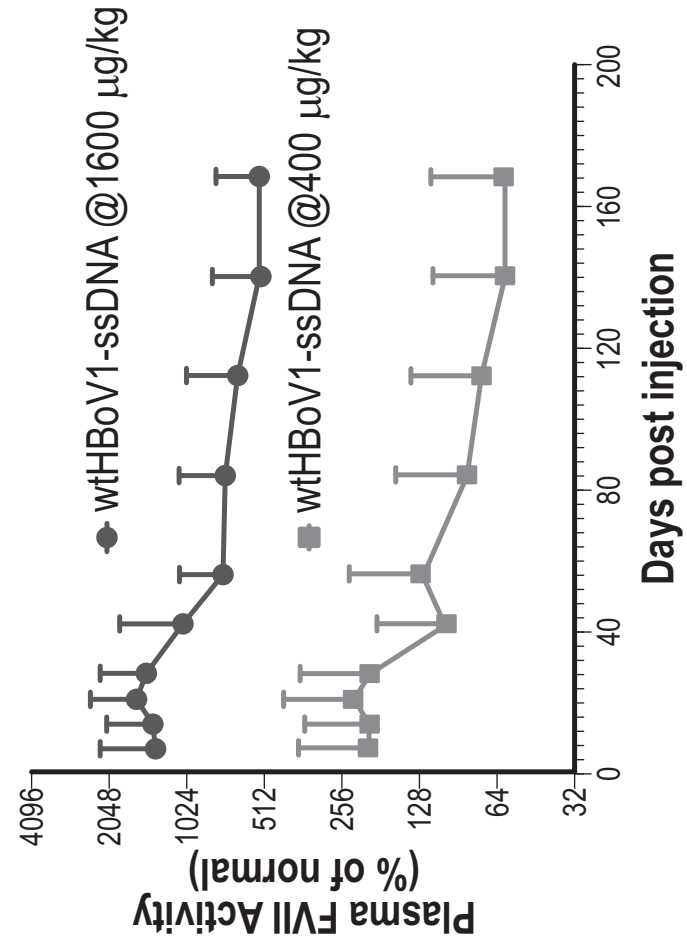
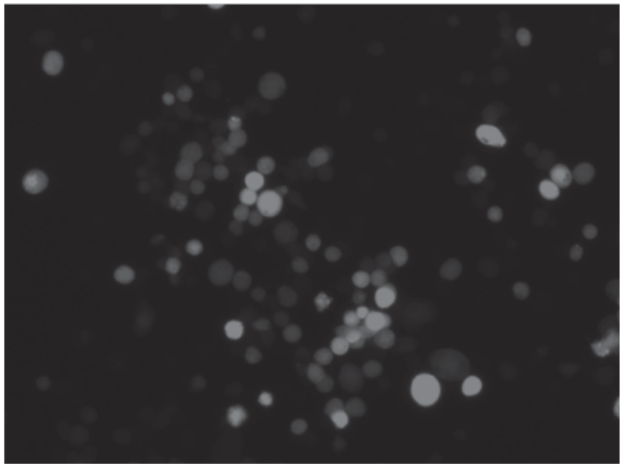


FIG. 14A



AcBIVVBac.Polh.HBoV1.NS1^{Tn7}
+ Cas9 alone



AcBIVVBac.Polh.HBoV1.NS1^{Tn7}
+ sgRNA.VP80.T1



AcBIVVBac.Polh.HBoV1.NS1^{Tn7}
+ sgRNA.VP80.T2

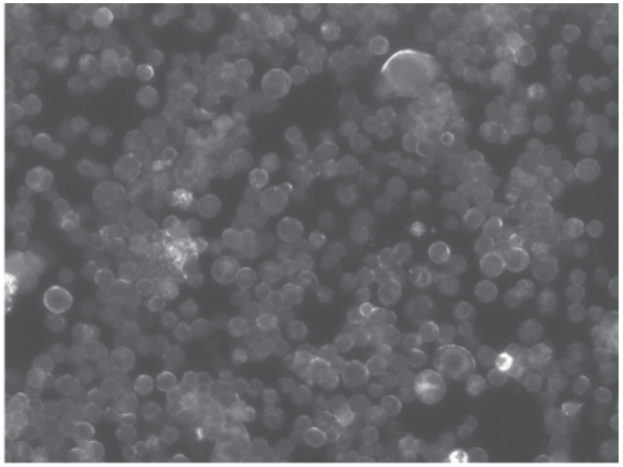


FIG. 15A

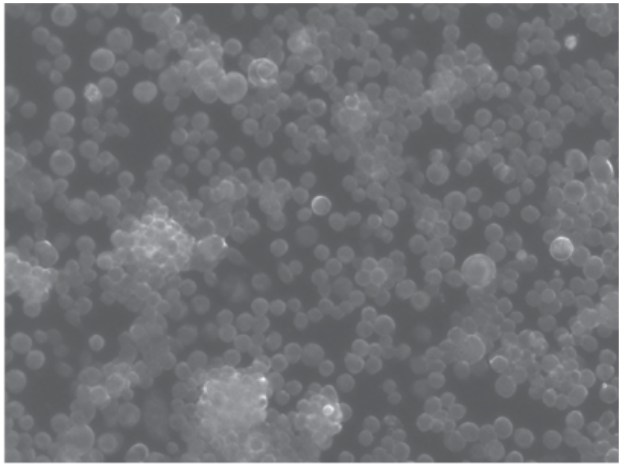


FIG. 15B

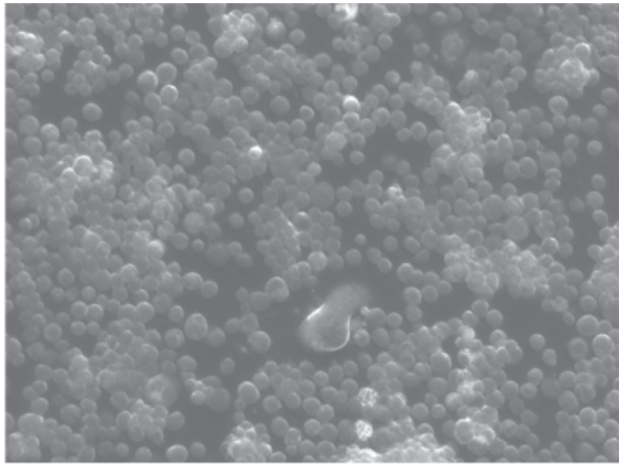


FIG. 15C

FIG. 16A

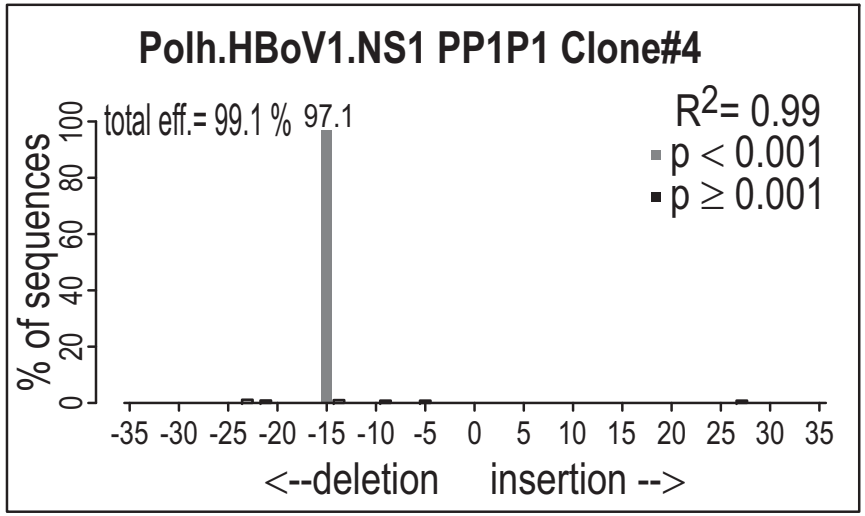


FIG. 16B

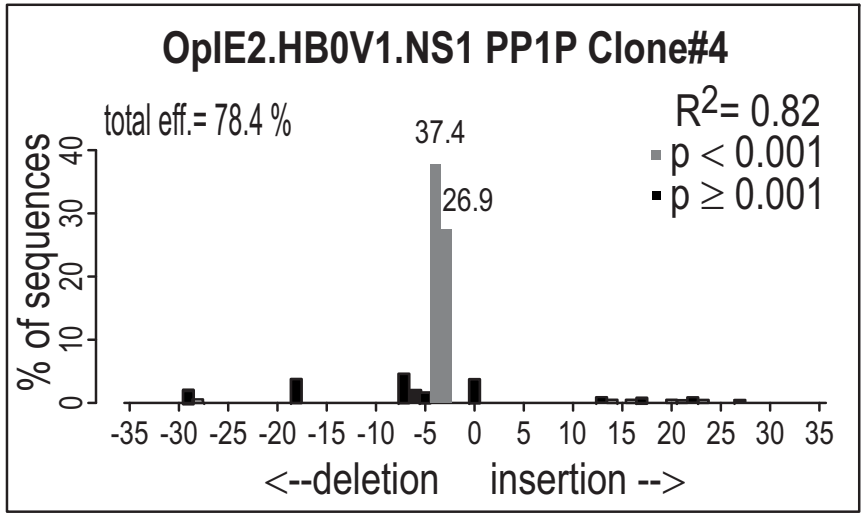
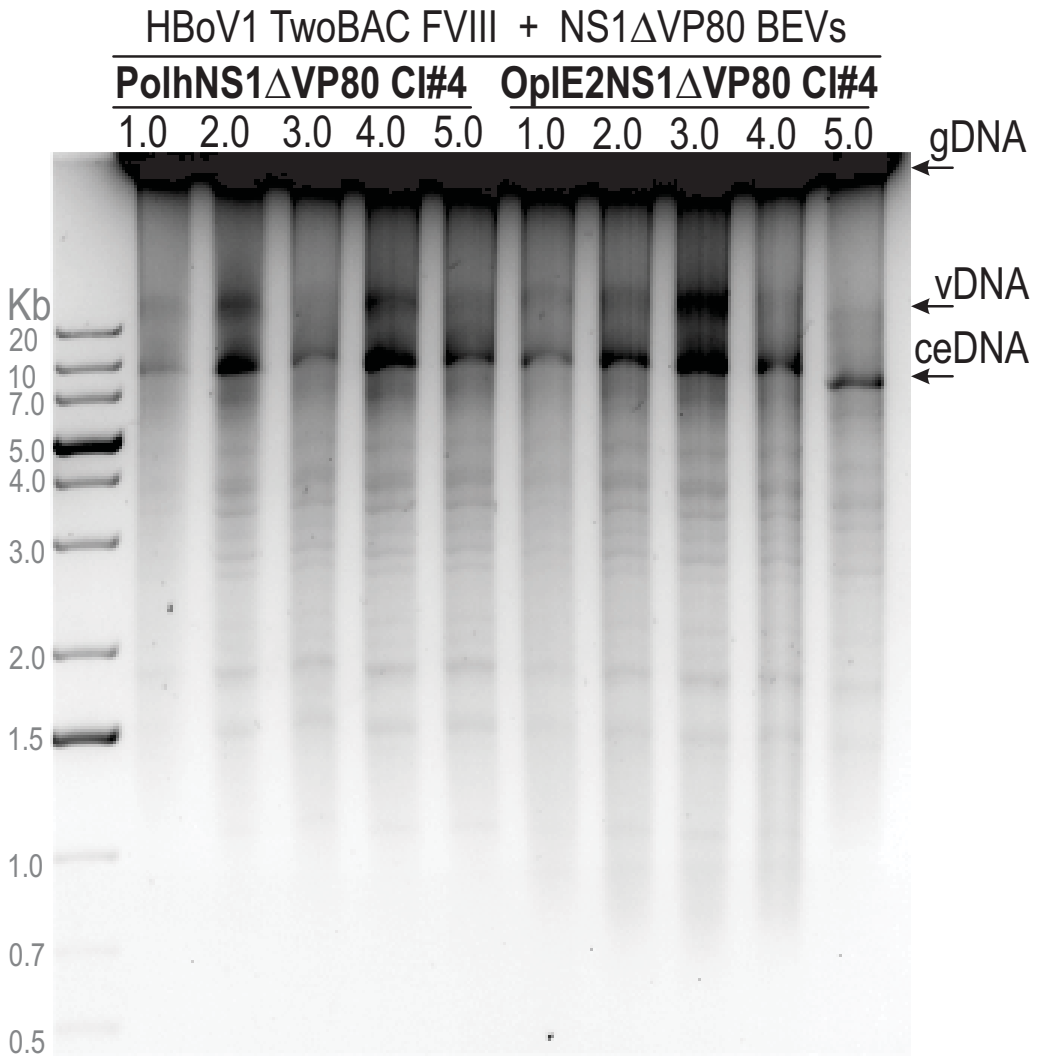


FIG. 16C



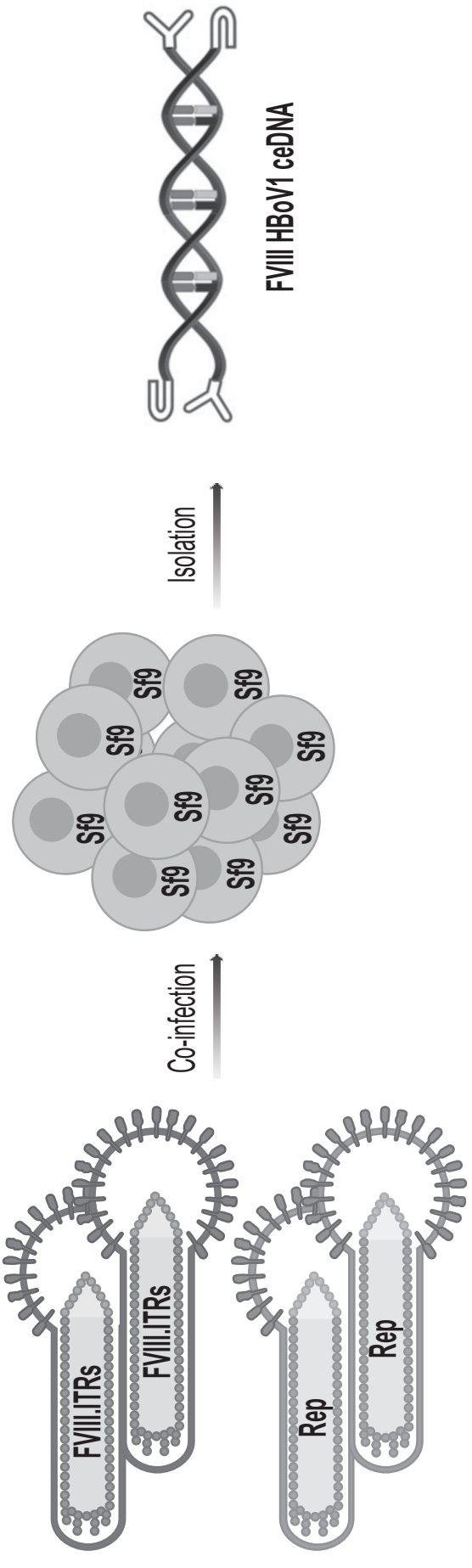

FVIII.ITRs
FVIII.ITRs
Rep
Rep
Co-infection
Sf9
Isolation
FVIII HBoV1 ceDNA

FIG. 17A

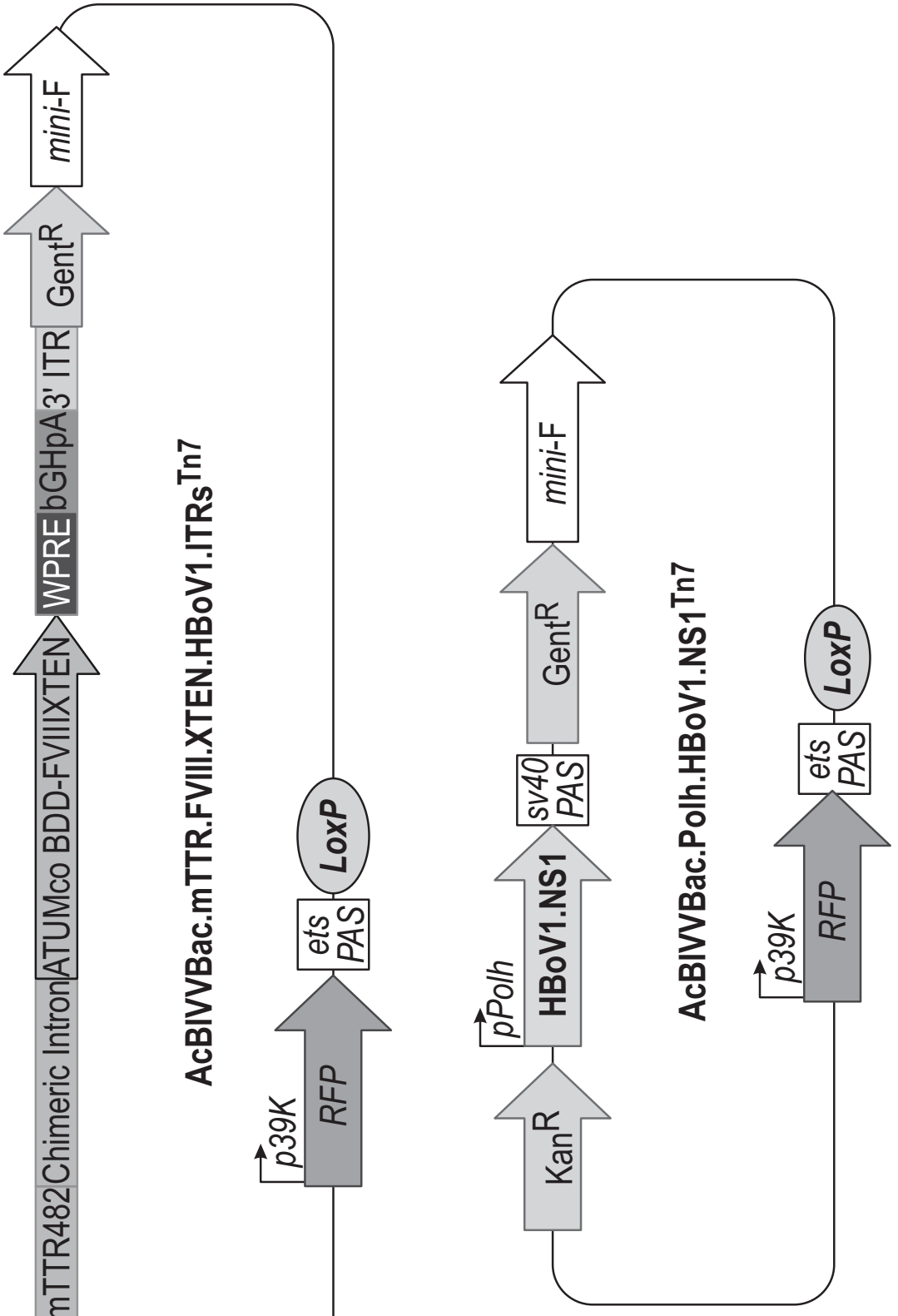

Kan^R
5' ITR
A1MB2
mTTR482
Chimeric Intron
ATUMco BDD-FVIIIXTEN
WPRE
bGHpA
3' ITR
Gent^R
mini-F
AcBIVVBac.mTTR.FVIII.XTEN.HBoV1.ITRs^Tn7
p39K
RFP
ets
PAS
LoxP
Kan^R
pPolh
HBoV1.NS1
sv40
PAS
Gent^R
mini-F
AcBIVVBac.Polh.HBoV1.NS1^Tn7
p39K
RFP
ets
PAS
LoxP

FIG. 17B

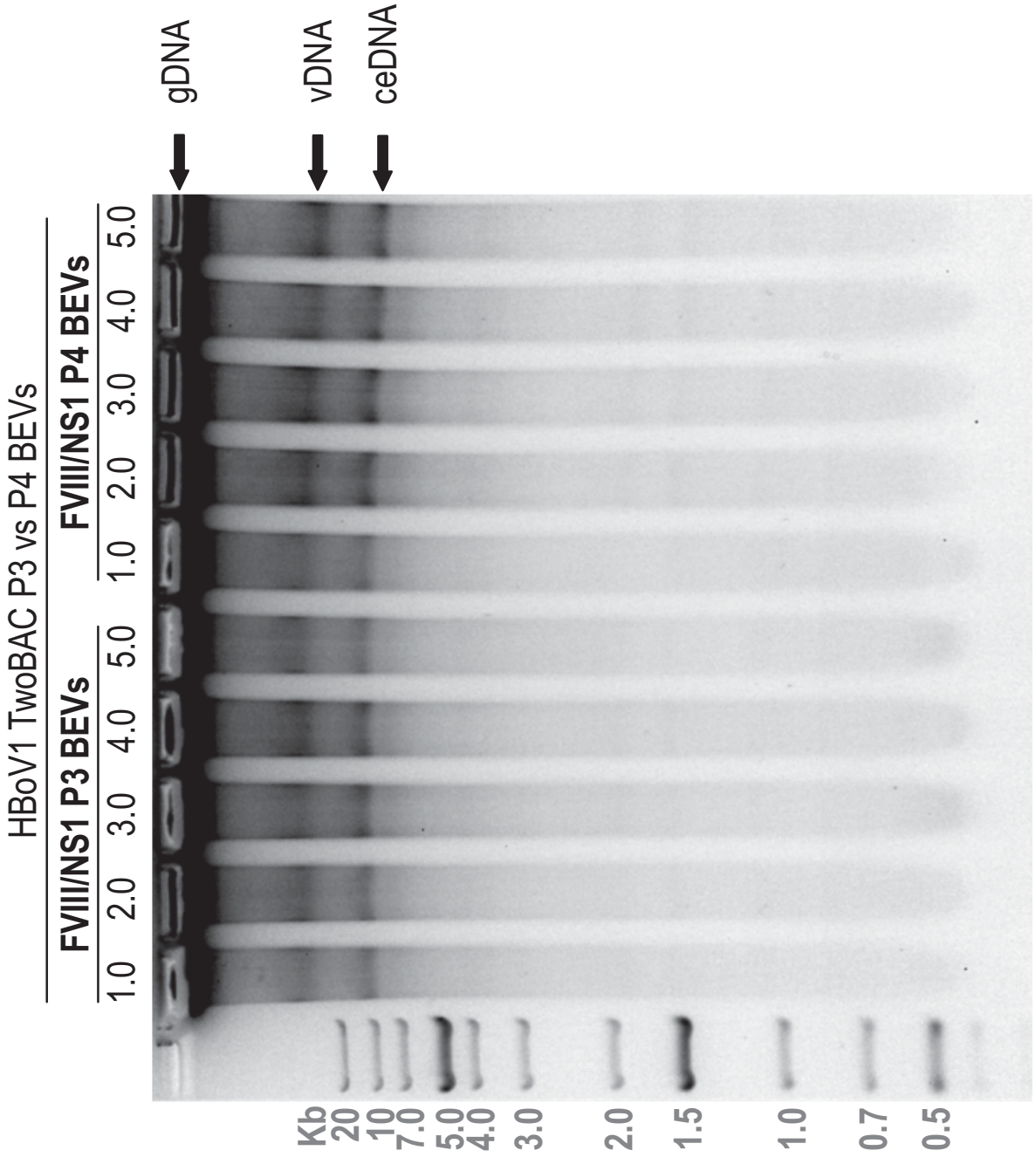

HBoV1 TwoBAC P3 vs P4 BEVs
FVIII/NS1 P3 BEVs
FVIII/NS1 P4 BEVs
1.0
2.0
3.0
4.0
5.0
1.0
2.0
3.0
4.0
5.0
gDNA
vDNA
ceDNA
Kb
20
10
7.0
5.0
4.0
3.0
2.0
1.5
1.0
0.7
0.5

FIG. 17C

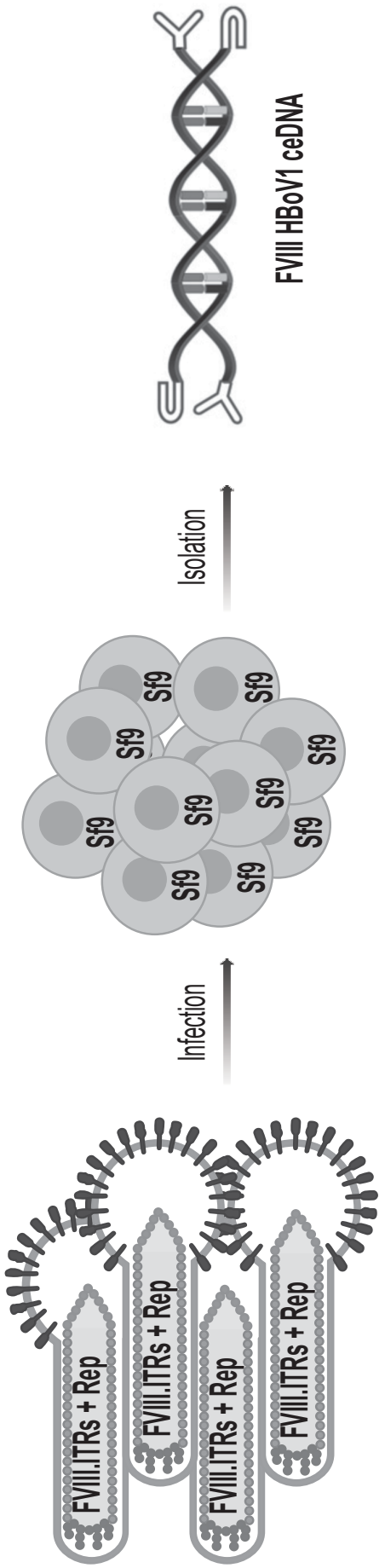


FIG. 18A

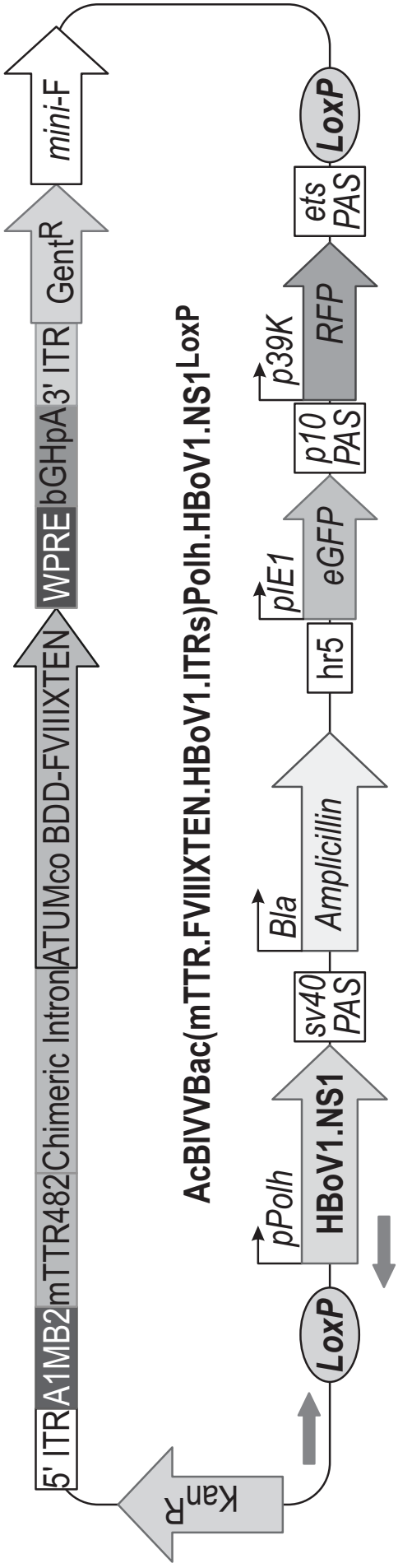


FIG. 18B

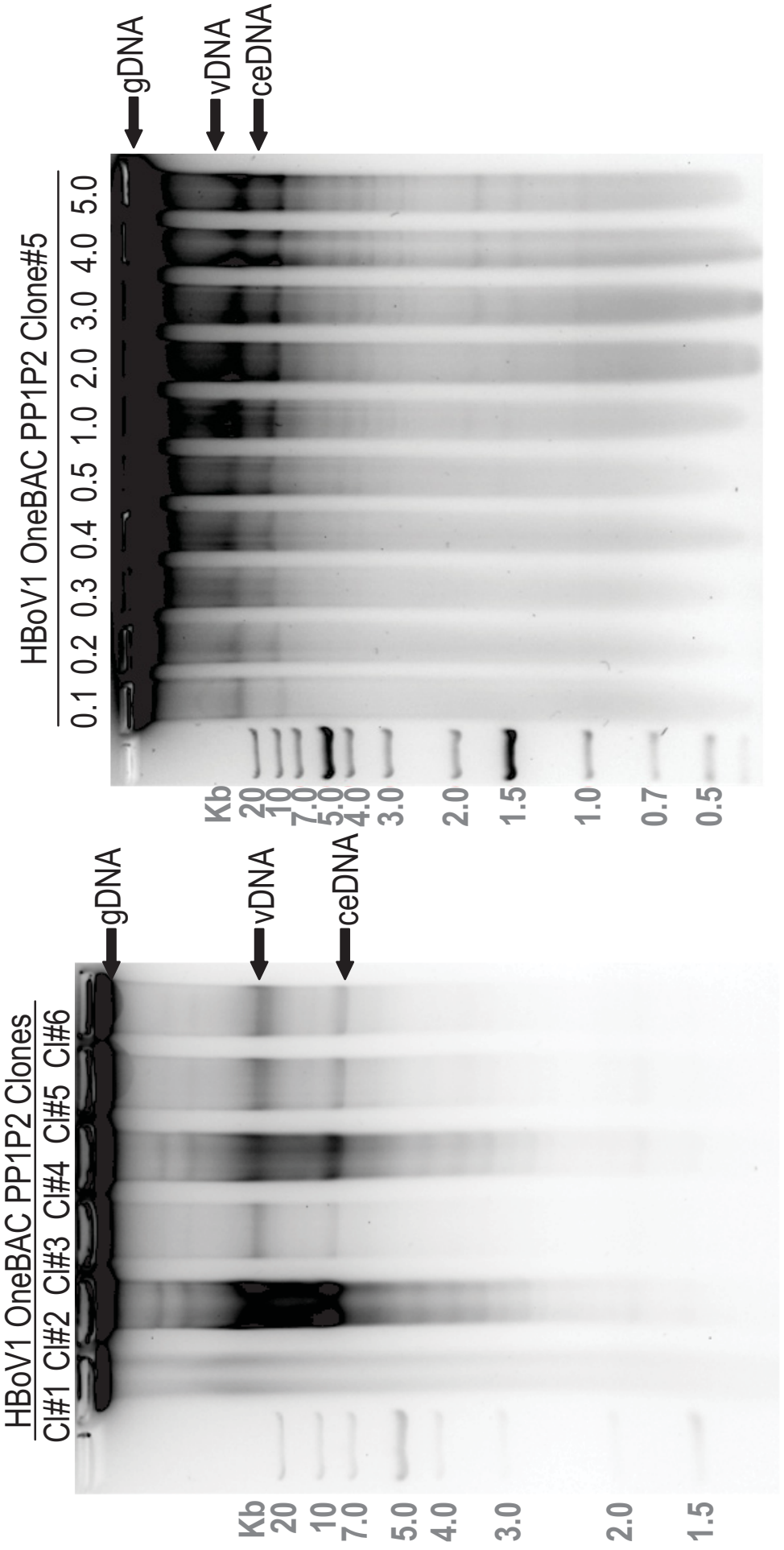


FIG. 18D

FIG. 18C

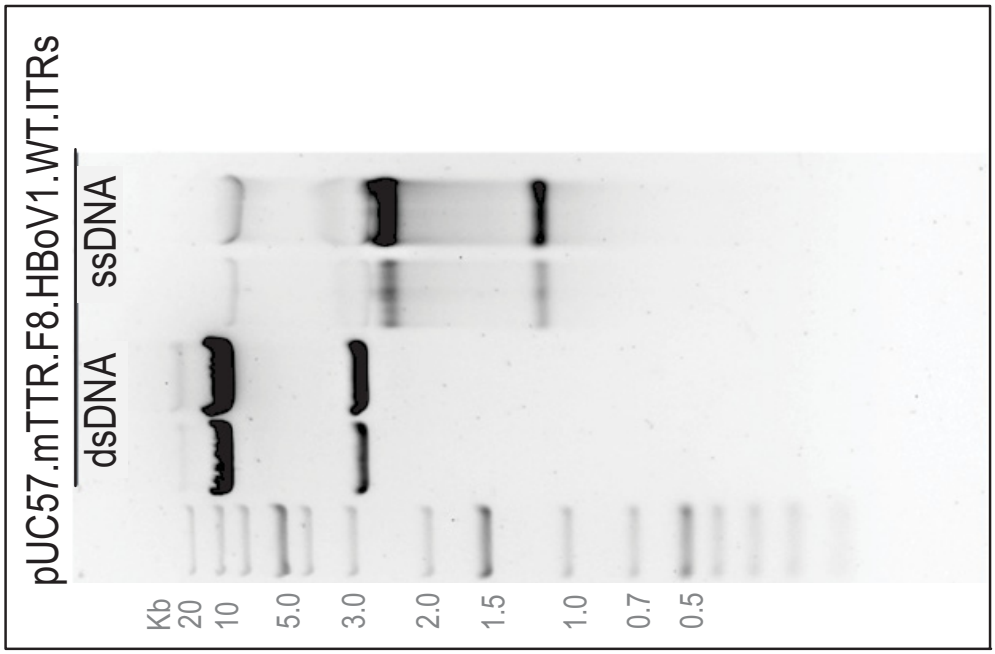


FIG. 19A

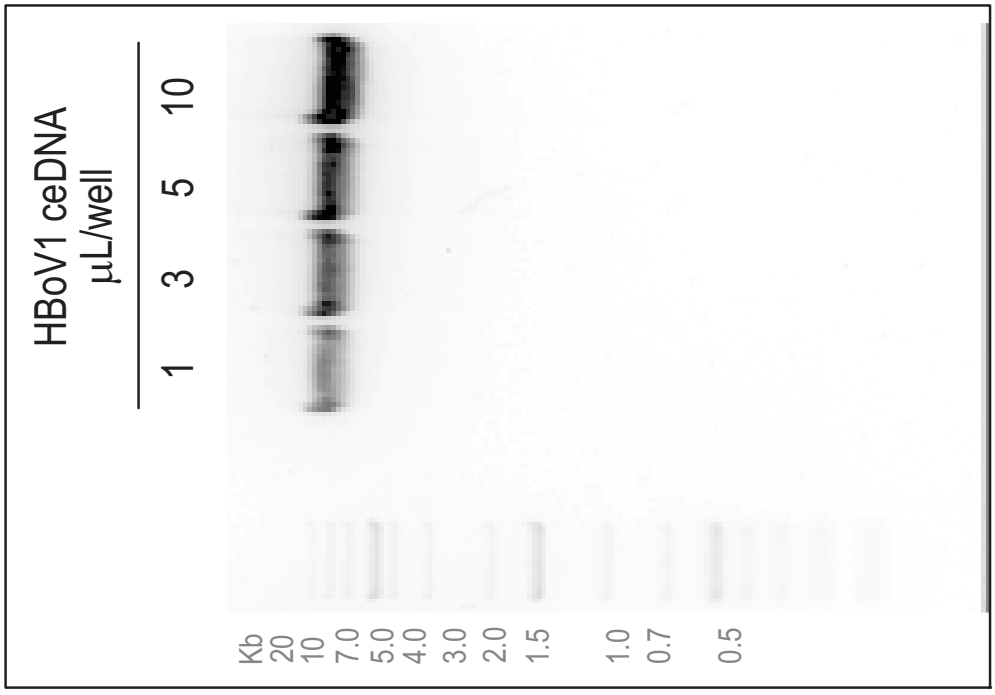


FIG. 19B

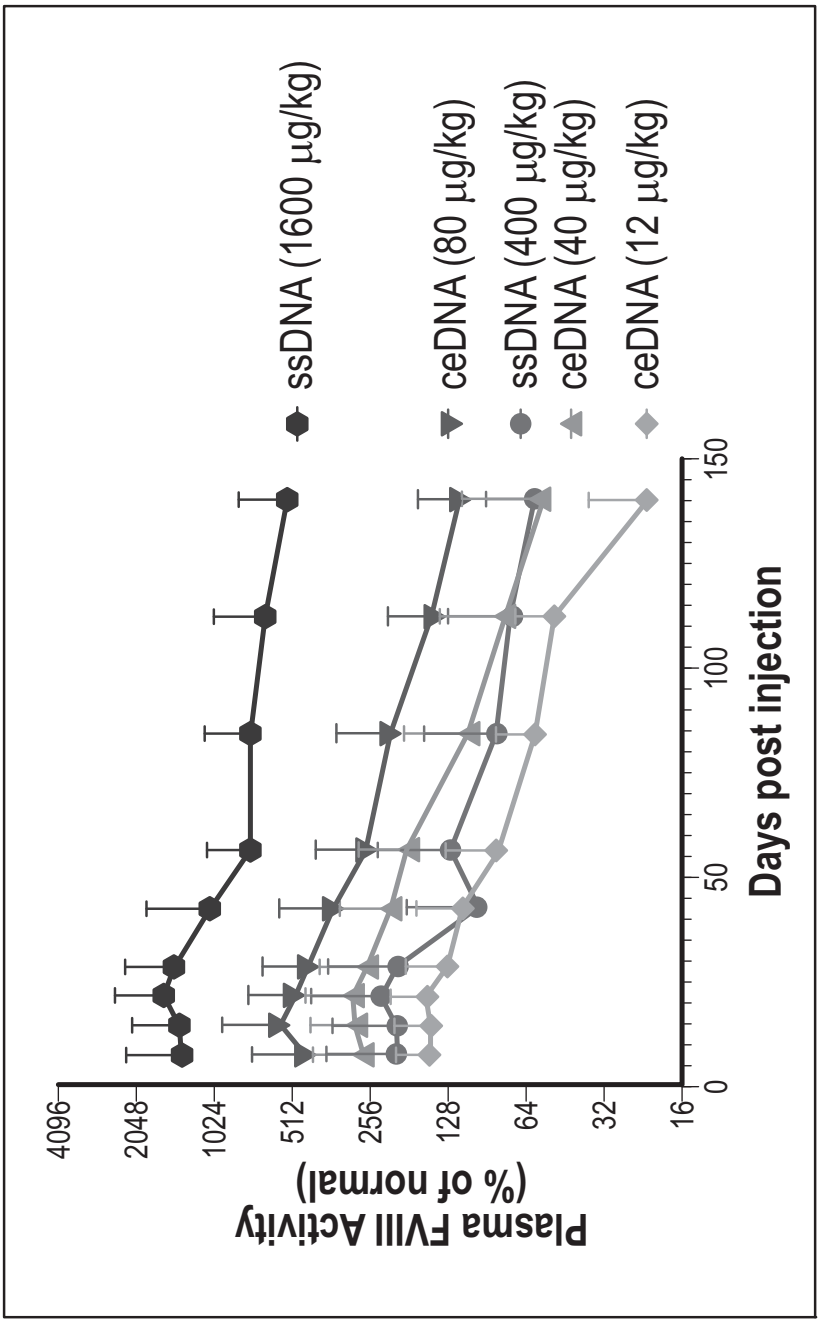

Plasma FVIII Activity
(% of normal)
4096
2048
1024
512
256
128
64
32
16
0
50
100
150
Days post injection
ssDNA (1600 µg/kg)
ceDNA (80 µg/kg)
ssDNA (400 µg/kg)
ceDNA (40 µg/kg)
ceDNA (12 µg/kg)

FIG. 19C

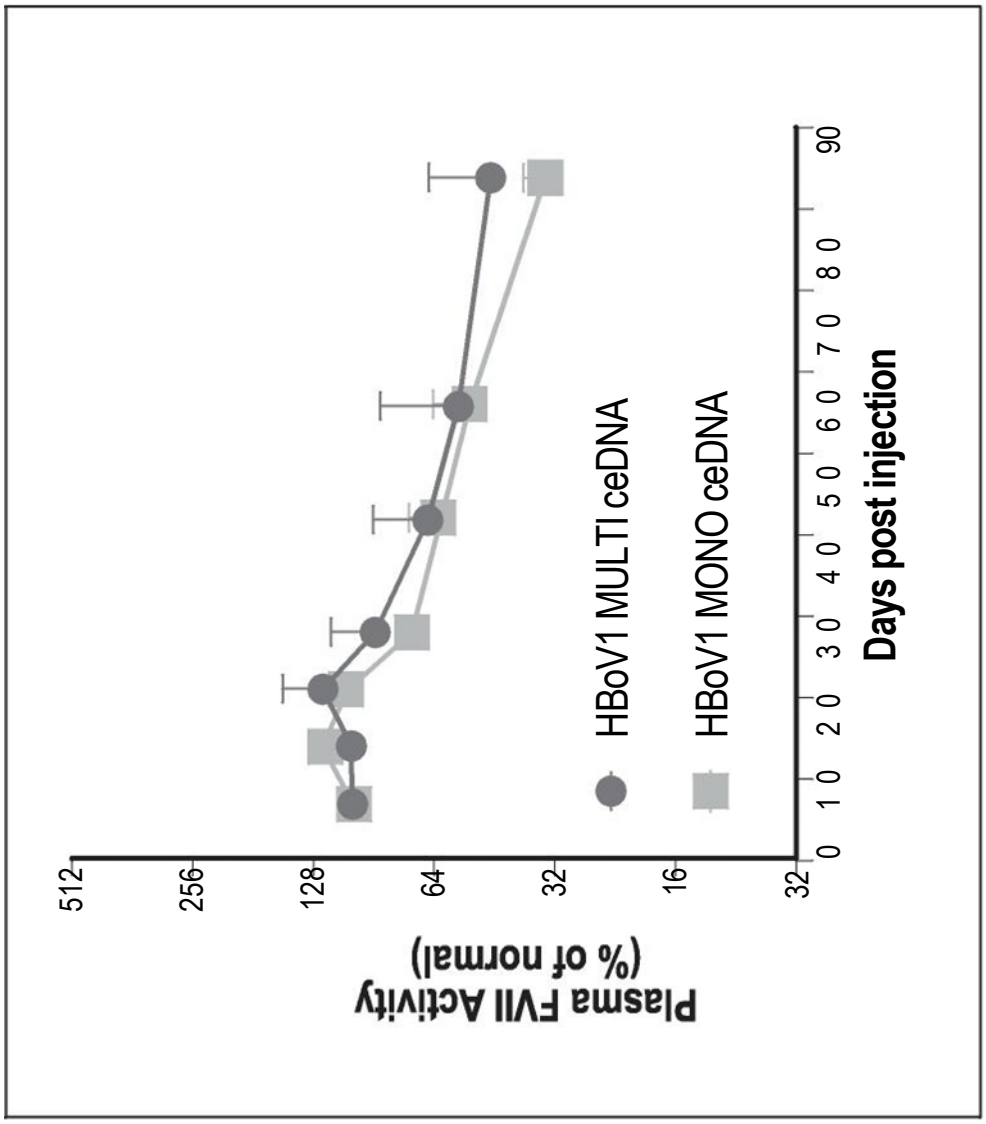


FIG. 20B

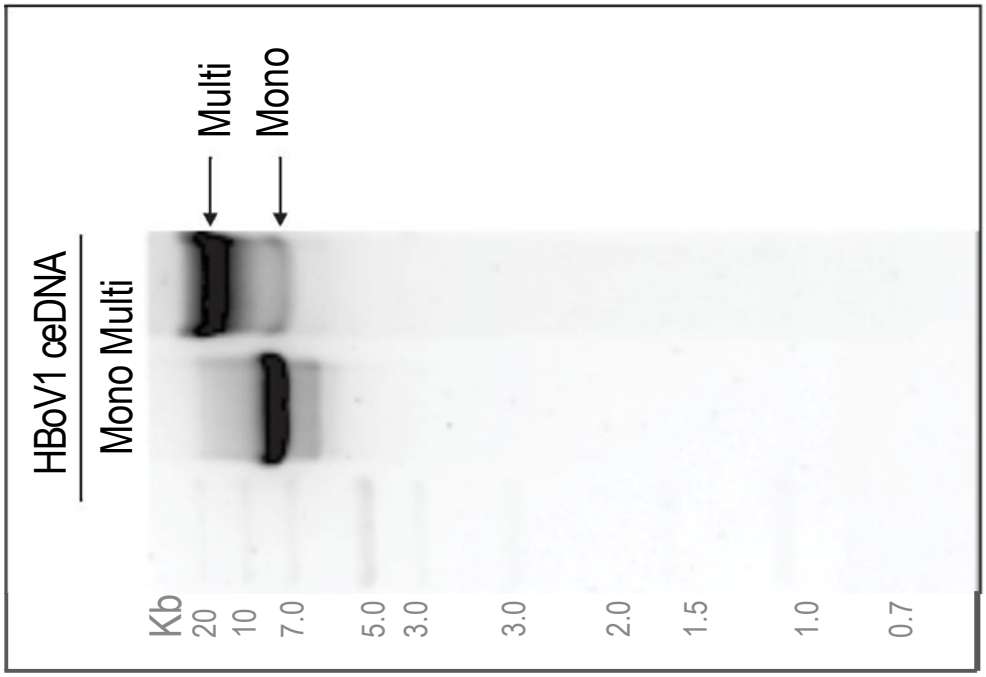


FIG. 20A

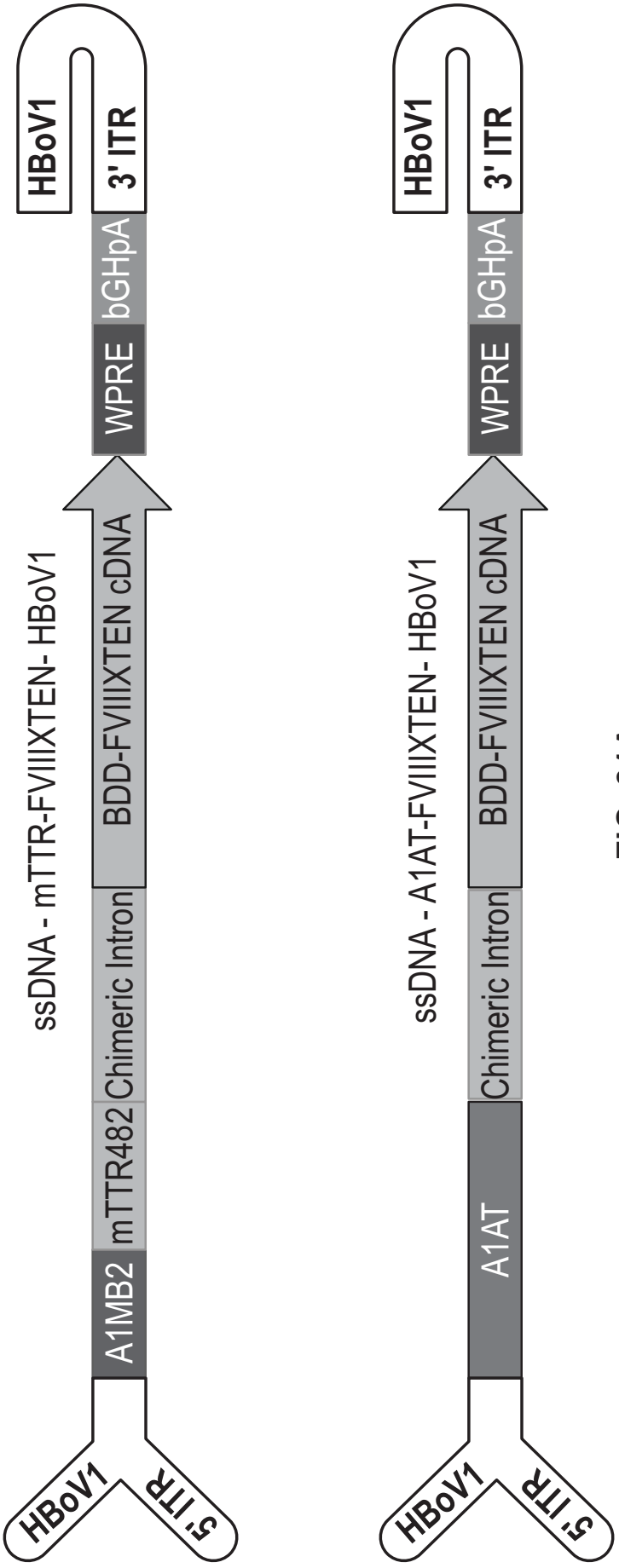

ssDNA - mTTR-FVIIIXTEN- HBoV1
HBoV1
5' ITR
A1MB2
mTTR482
Chimeric Intron
BDD-FVIIIXTEN cDNA
WPRE
bGHpA
3' ITR
HBoV1
ssDNA - A1AT-FVIIIXTEN- HBoV1
HBoV1
5' ITR
A1AT
Chimeric Intron
BDD-FVIIIXTEN cDNA
WPRE
bGHpA
3' ITR
HBoV1

FIG. 21A

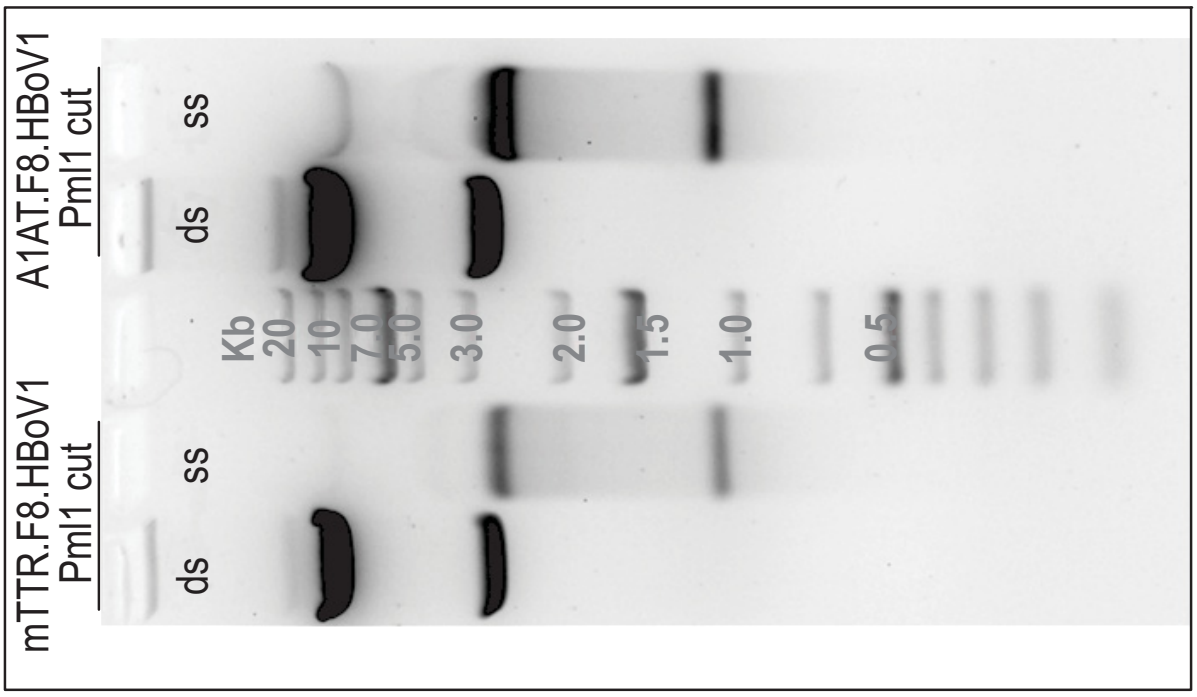


FIG. 21B

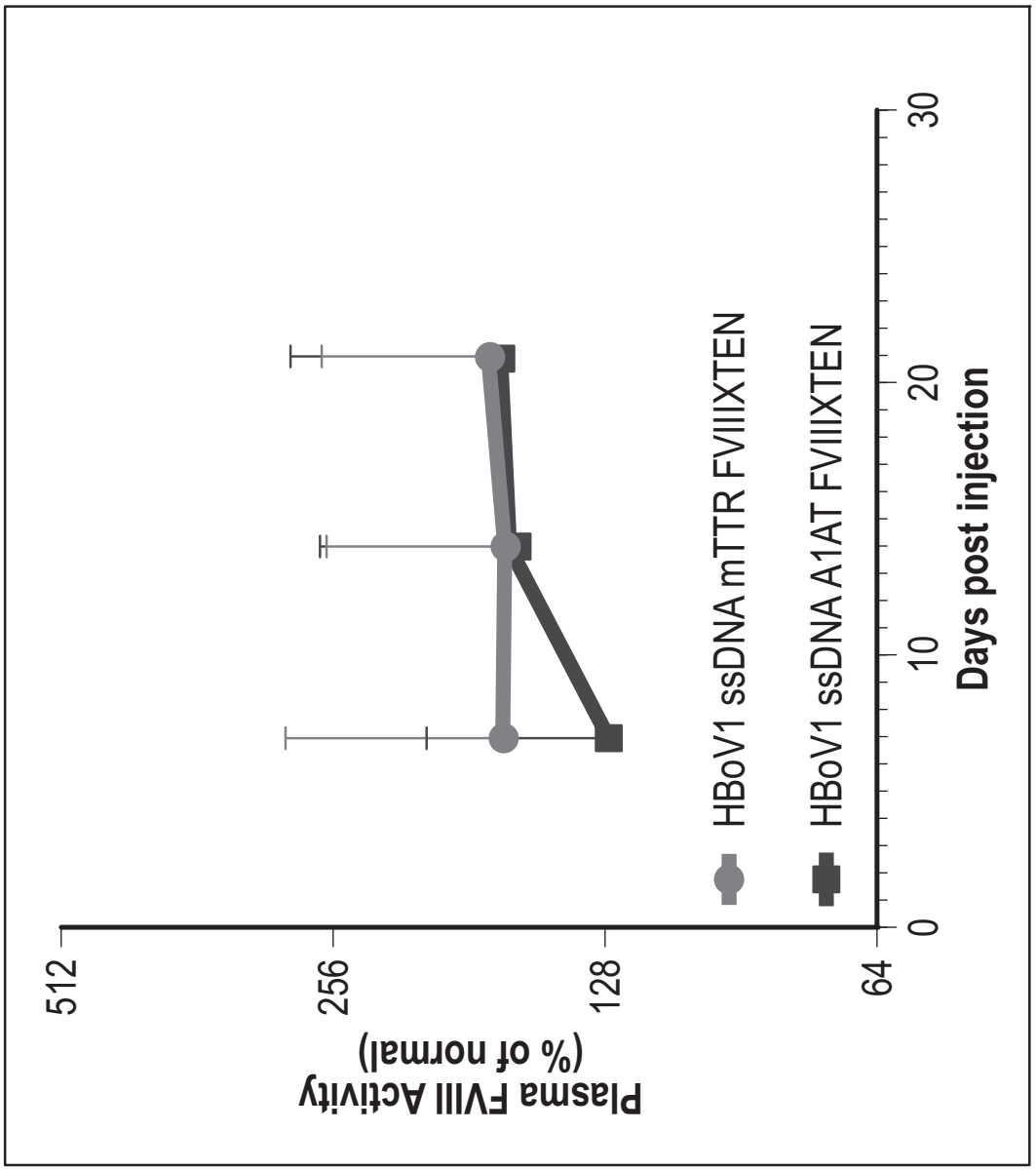


FIG. 21C