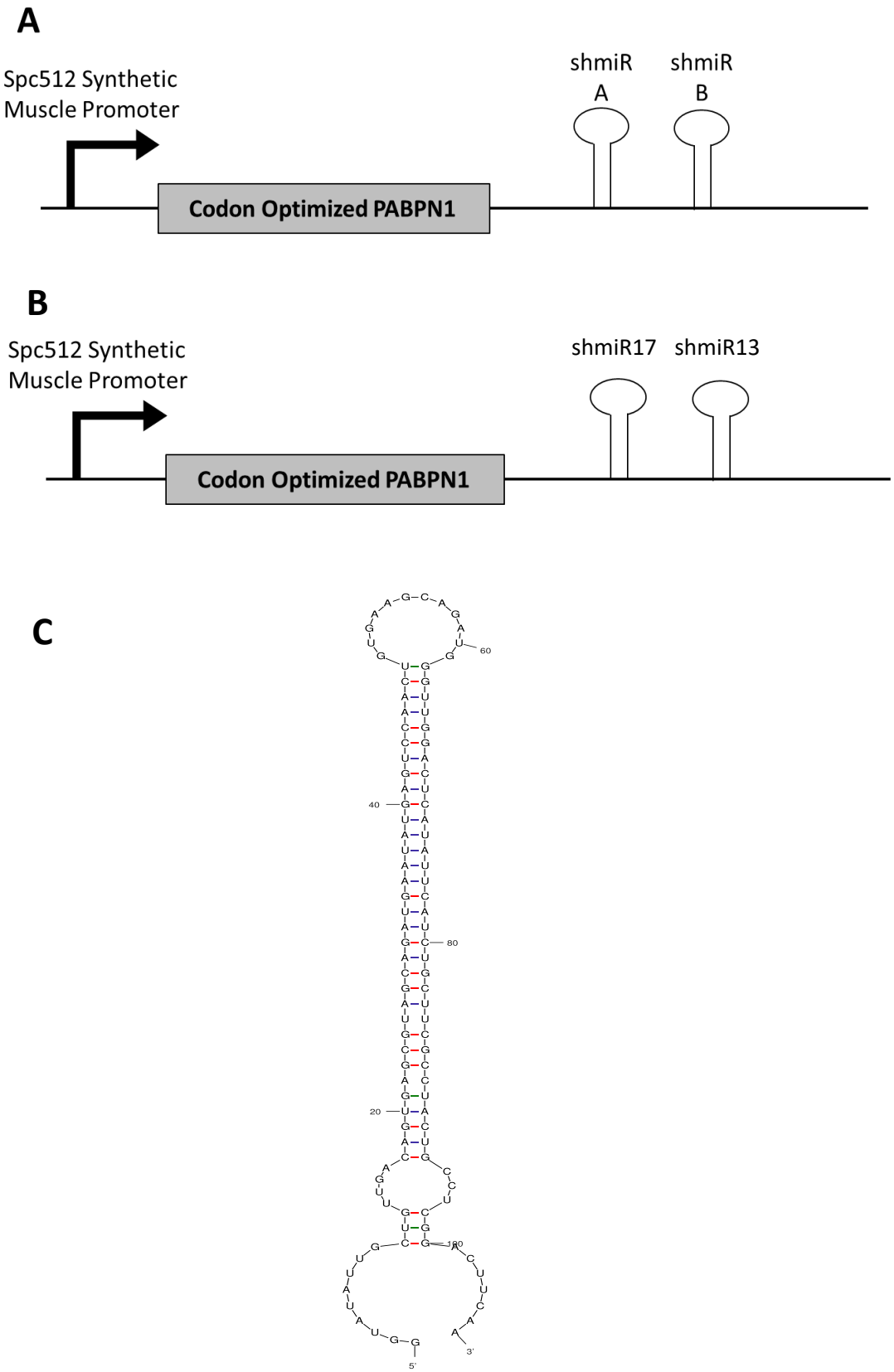

A
Spc512 Synthetic
Muscle Promoter
shmiR
A
shmiR
B
Codon Optimized PABPN1
B
Spc512 Synthetic
Muscle Promoter
shmiR17
shmiR13
Codon Optimized PABPN1
C
5'
3'

Figure 1

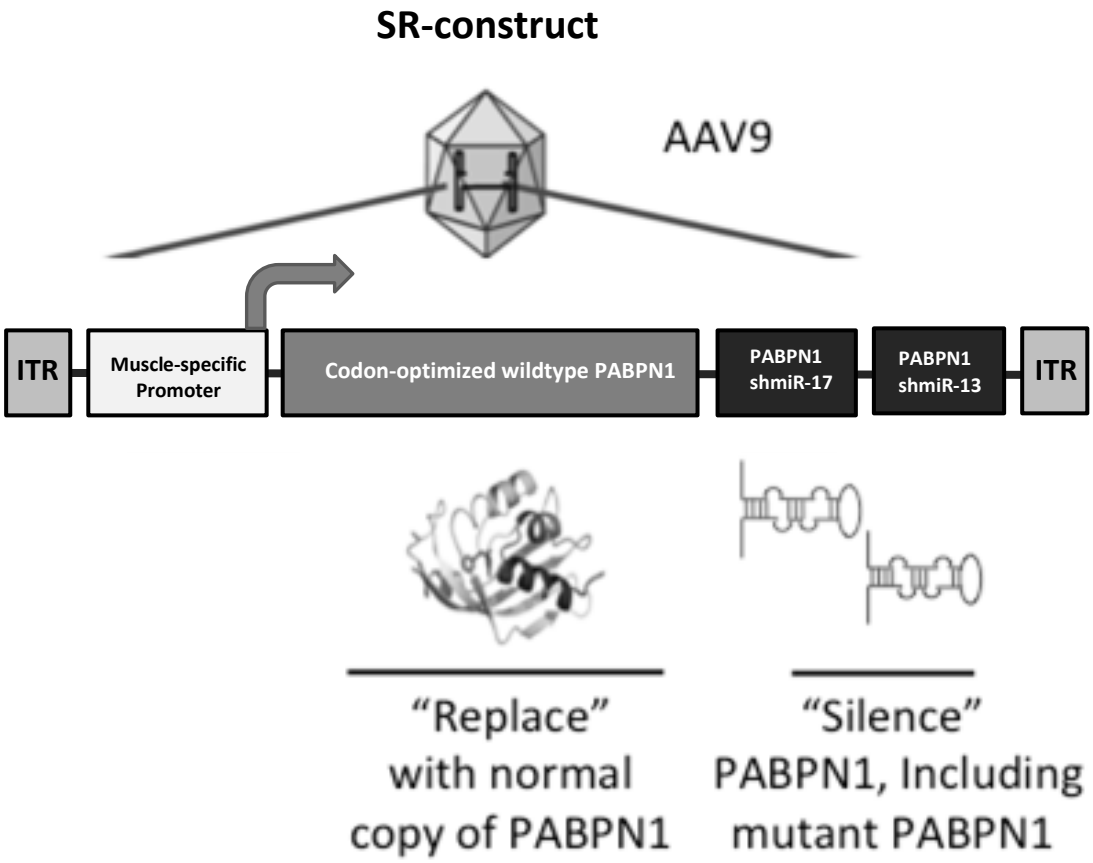

SR-construct
AAV9
ITR
Muscle-specific Promoter
Codon-optimized wildtype PABPN1
PABPN1 shmiR-17
PABPN1 shmiR-13
ITR
"Replace" with normal copy of PABPN1
"Silence" PABPN1, Including mutant PABPN1

Figure 2

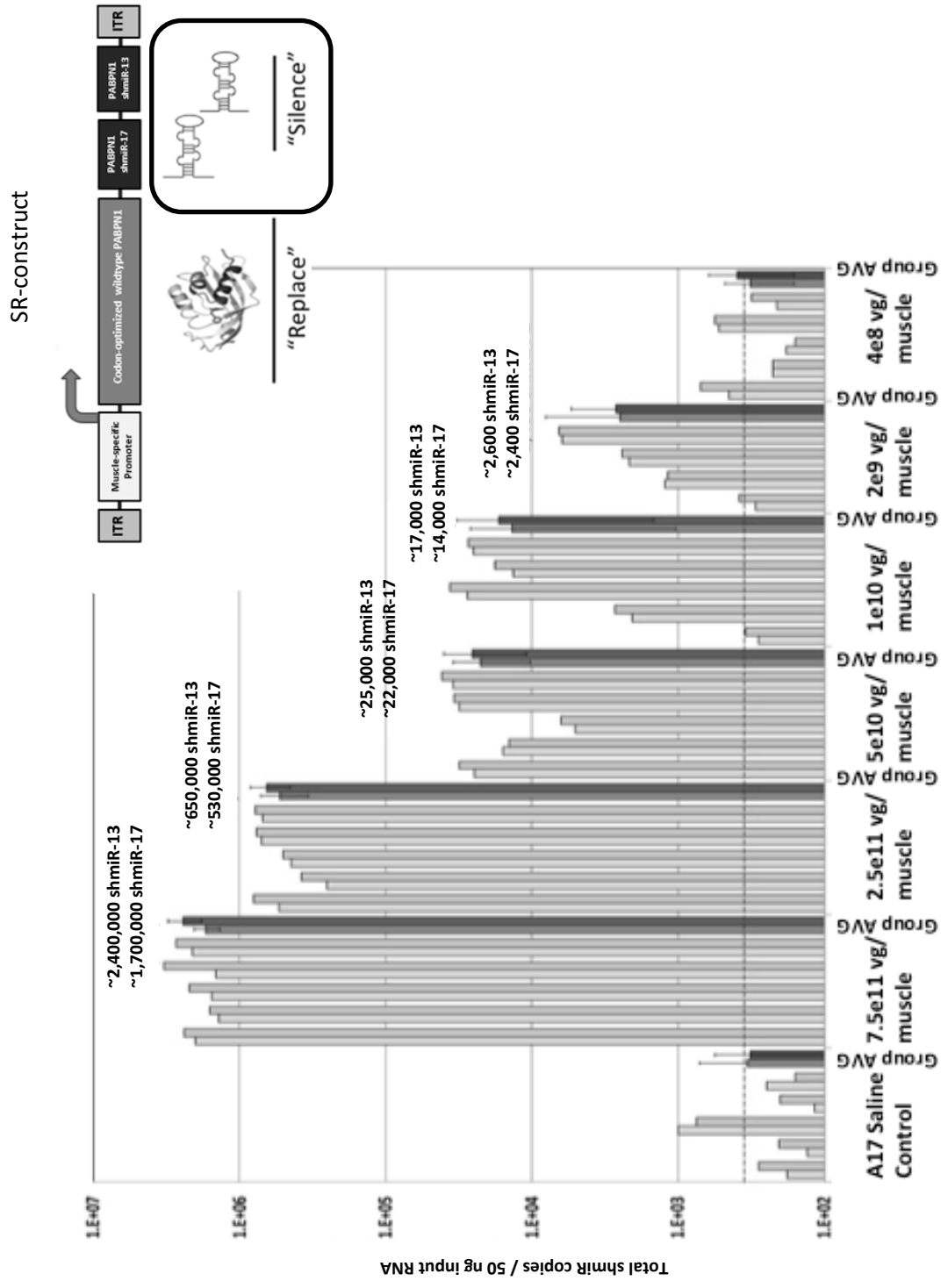

SR-construct
ITR
Muscle-specific Promoter
Codon-optimized wildtype PABPN1
PABPN1 shmiR-17
PABPN1 shmiR-13
ITR
"Replace"
"Silence"
~2,400,000 shmiR-13
~1,700,000 shmiR-17
~650,000 shmiR-13
~530,000 shmiR-17
~25,000 shmiR-13
~22,000 shmiR-17
~17,000 shmiR-13
~14,000 shmiR-17
~2,600 shmiR-13
~2,400 shmiR-17
Total shmiR copies / 50 ng input RNA
1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
A17 Saline Control
Group AVG
7.5e11 vg/ muscle
2.5e11 vg/ muscle
5e10 vg/ muscle
1e10 vg/ muscle
2e9 vg/ muscle
4e8 vg/ muscle

Figure 3A

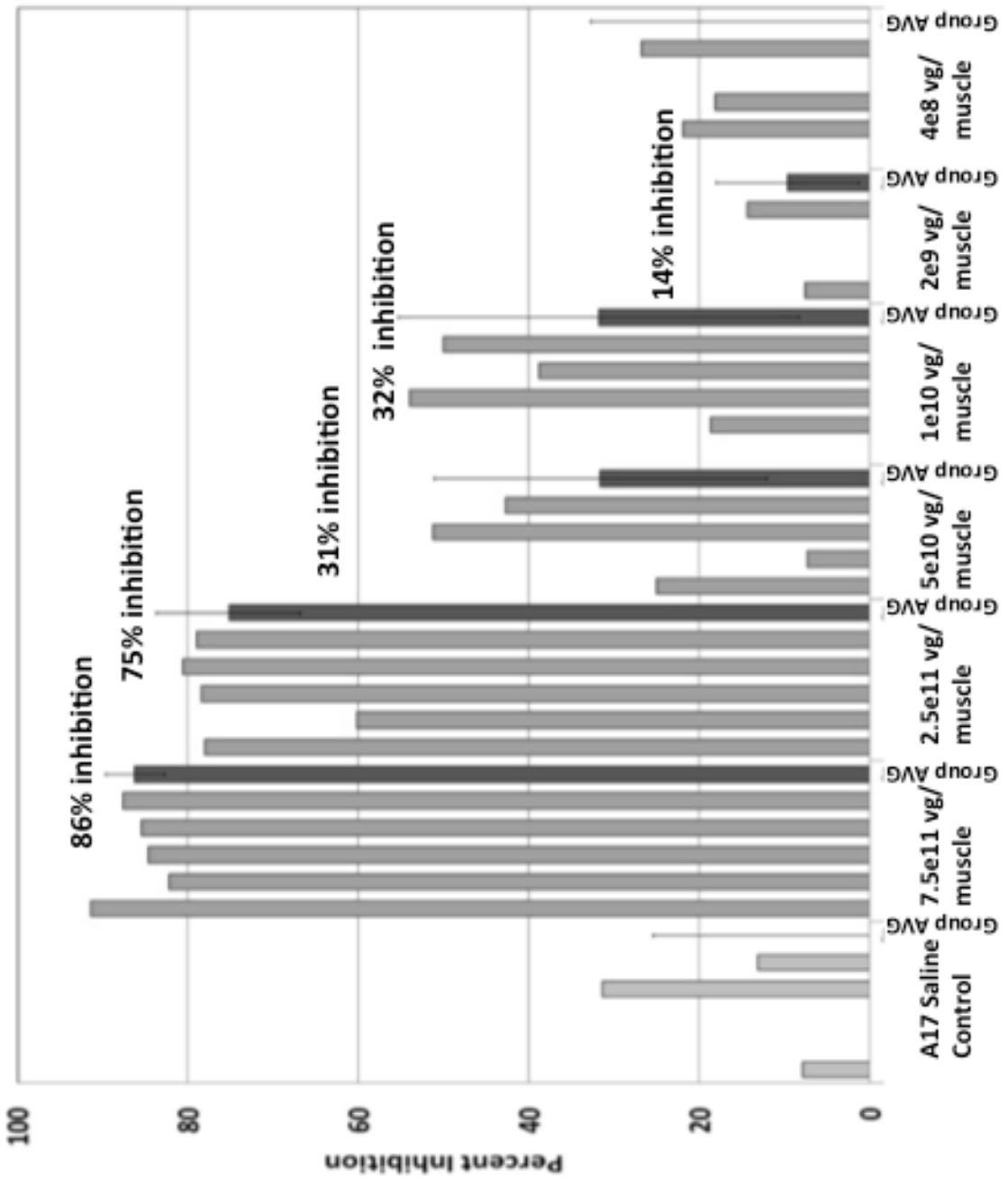

Percent Inhibition
100
80
60
40
20
0
A17 Saline Control
Group AVG
7.5e11 vg/ muscle
Group AVG
2.5e11 vg/ muscle
Group AVG
5e10 vg/ muscle
Group AVG
1e10 vg/ muscle
Group AVG
2e9 vg/ muscle
Group AVG
4e8 vg/ muscle
Group AVG
86% inhibition
75% inhibition
31% inhibition
32% inhibition
14% inhibition

Figure 3B

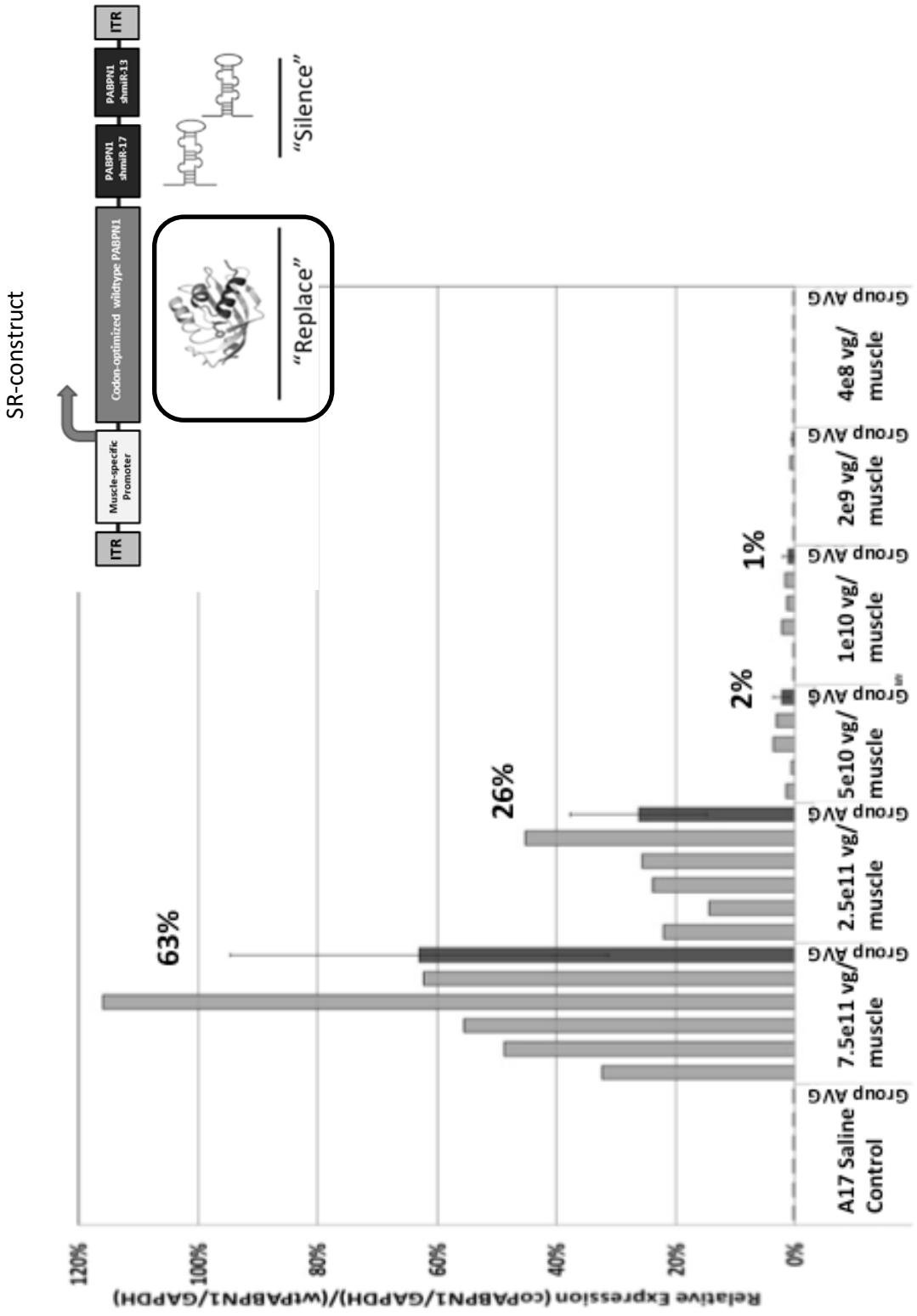

SR-construct
ITR
Muscle-specific Promoter
Codon-optimized wildtype PABPN1
PABPN1 shmiR-17
PABPN1 shmiR-13
ITR
"Replace"
"Silence"
Relative Expression (coPABPN1/GAPDH)/(wtPABPN1/GAPDH)
120%
100%
80%
60%
40%
20%
0%
63%
26%
2%
1%
A17 Saline Control
7.5e11 vg/ muscle
2.5e11 vg/ muscle
5e10 vg/ muscle
1e10 vg/ muscle
2e9 vg/ muscle
4e8 vg/ muscle
Group AVG

Figure 3C

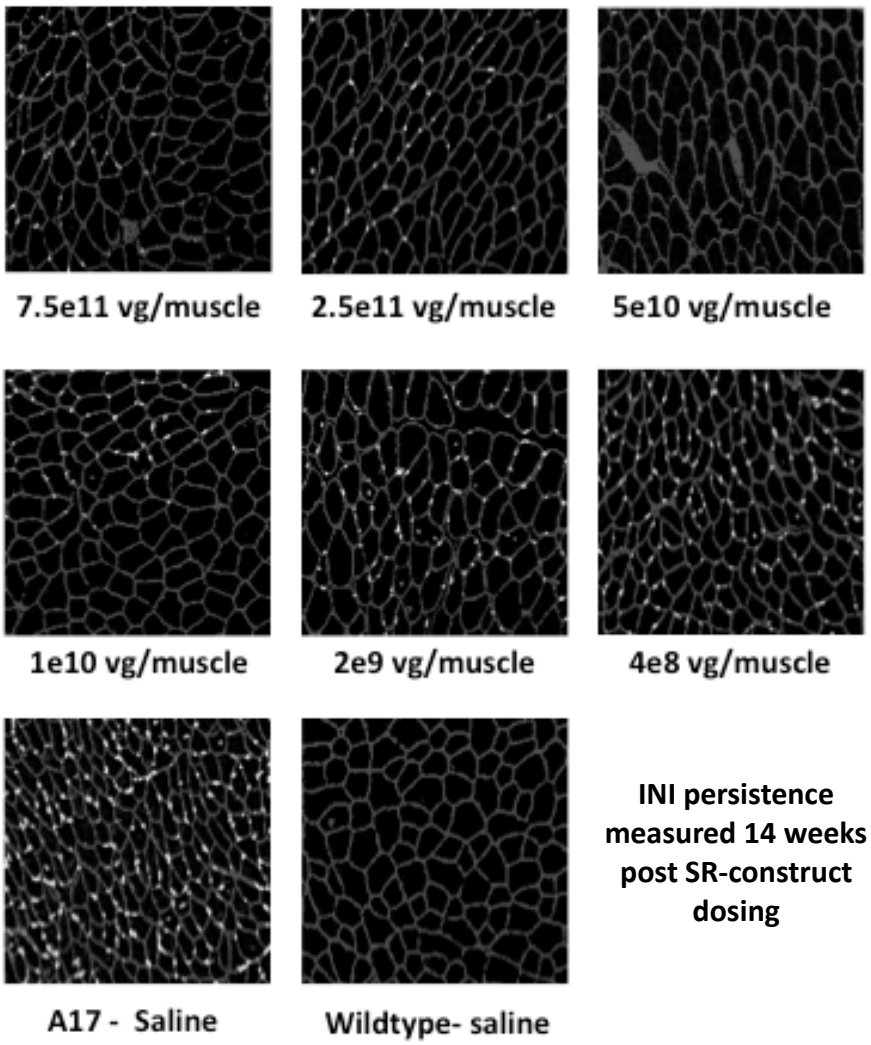

7.5e11 vg/muscle
2.5e11 vg/muscle
5e10 vg/muscle
1e10 vg/muscle
2e9 vg/muscle
4e8 vg/muscle
A17 - Saline
Wildtype- saline
INI persistence measured 14 weeks post SR-construct dosing

Figure 4A

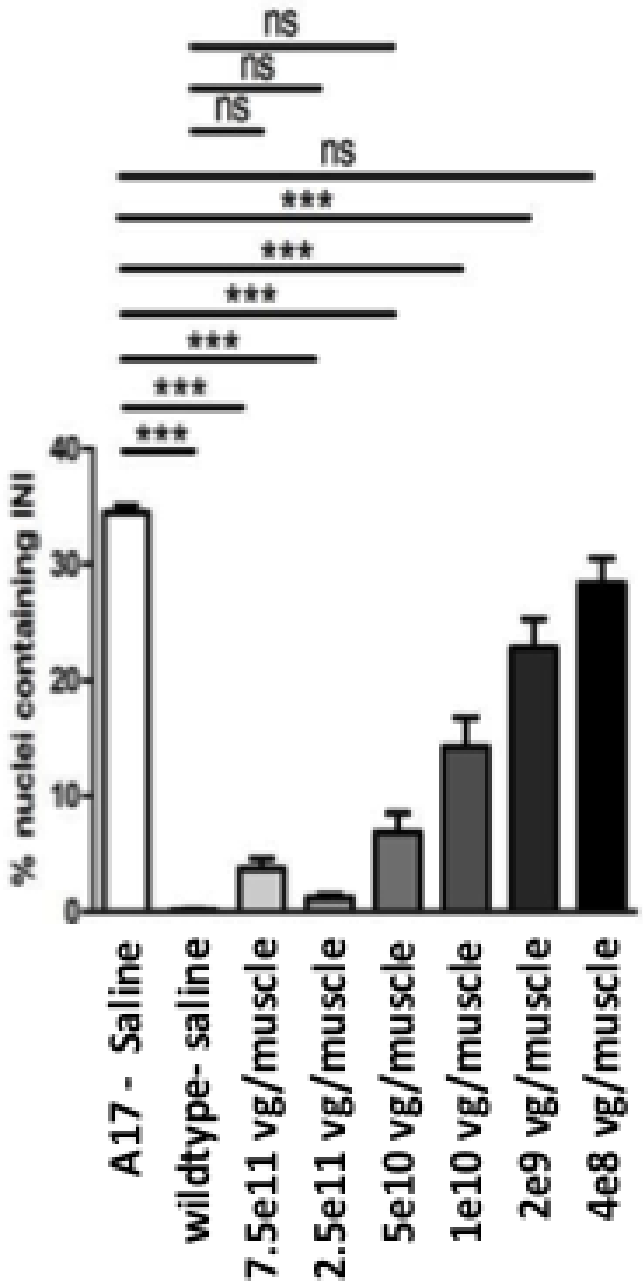

ns
ns
ns
ns
***
***
***
***
***
***
% nuclei containing INI
0
10
20
30
40
A17 - Saline
wildtype- saline
7.5e11 vg/muscle
2.5e11 vg/muscle
5e10 vg/muscle
1e10 vg/muscle
2e9 vg/muscle
4e8 vg/muscle

Figure 4B

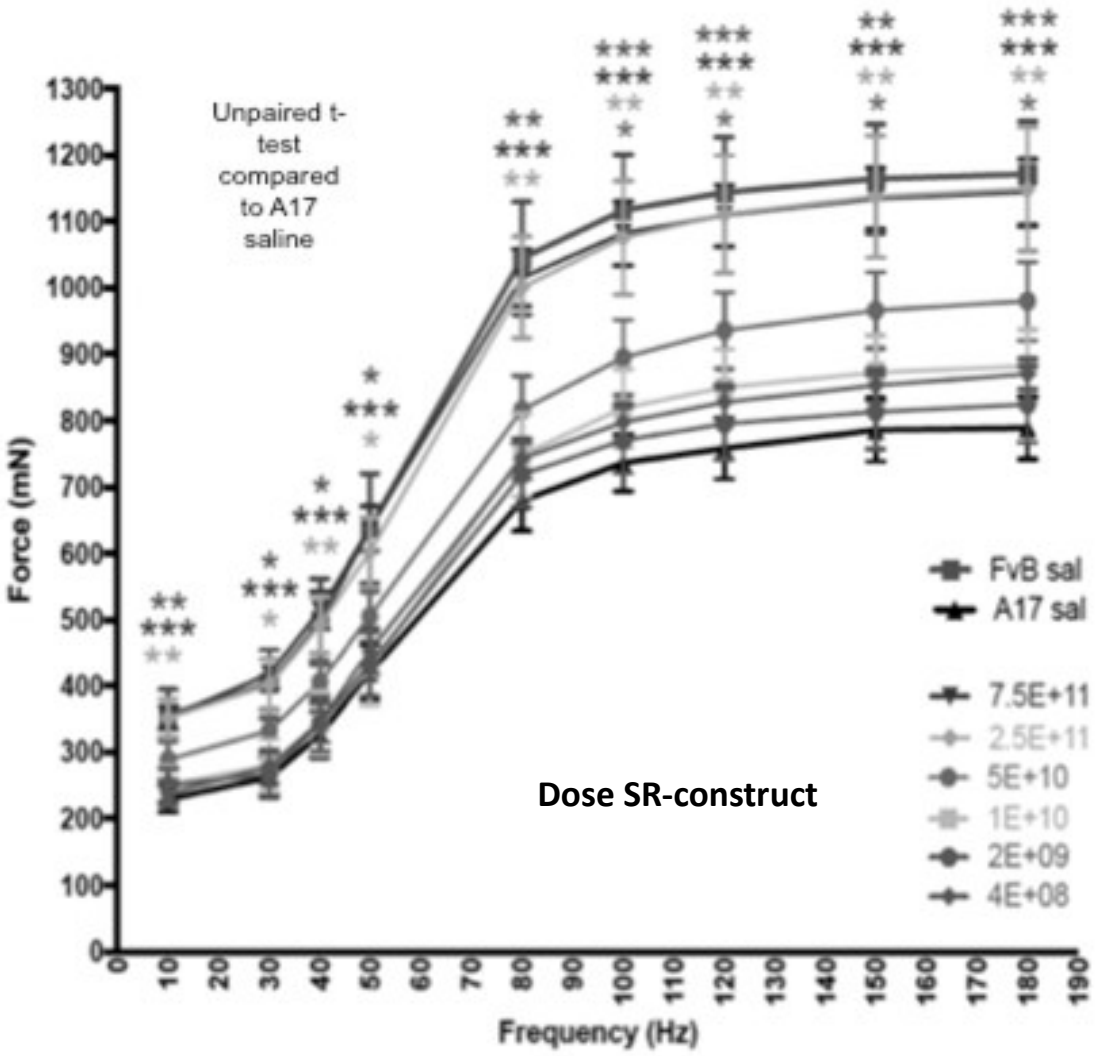

Unpaired t-test compared to A17 saline
Force (mN)
Frequency (Hz)
Dose SR-construct
FvB sal
A17 sal
7.5E+11
2.5E+11
5E+10
1E+10
2E+09
4E+08

Figure 5A

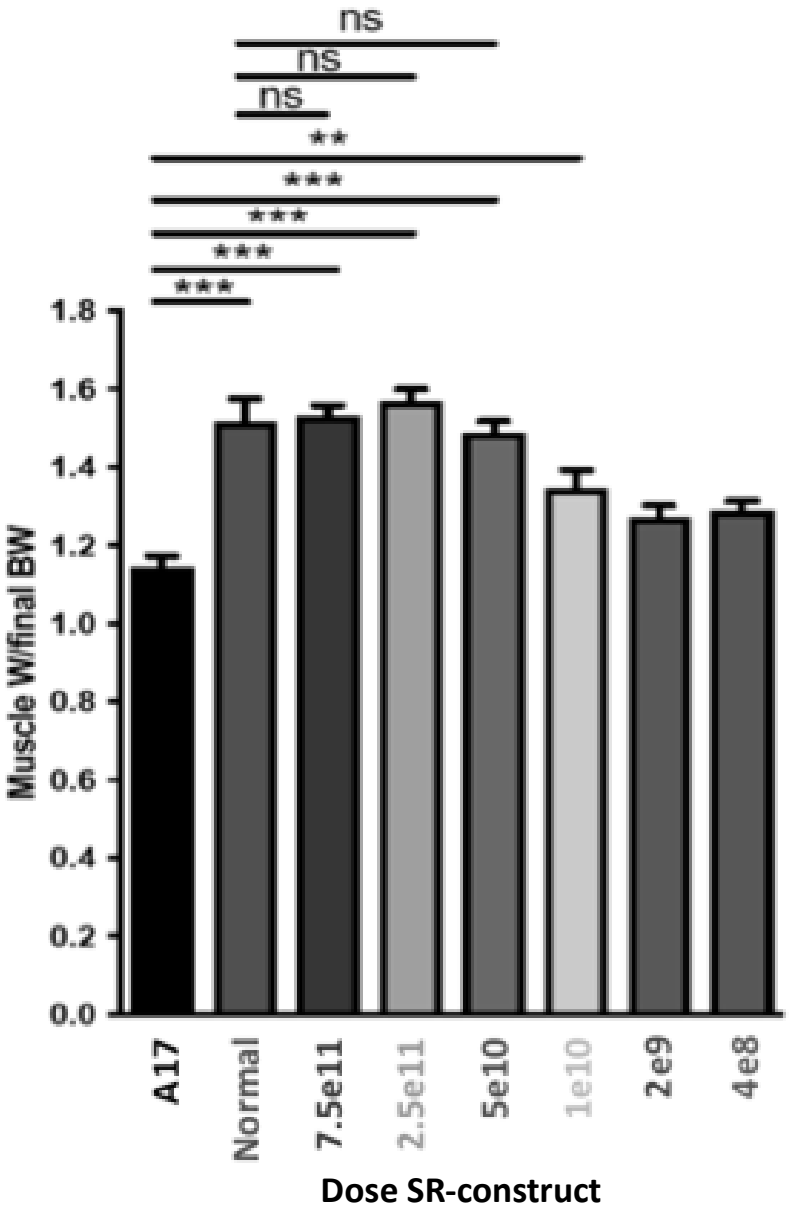

ns
ns
ns
**
***
***
***
***
Muscle W/final BW
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A17
Normal
7.5e11
2.5e11
5e10
1e10
2e9
4e8
Dose SR-construct

Figure 5B

14 weeks post SR-construct dosing

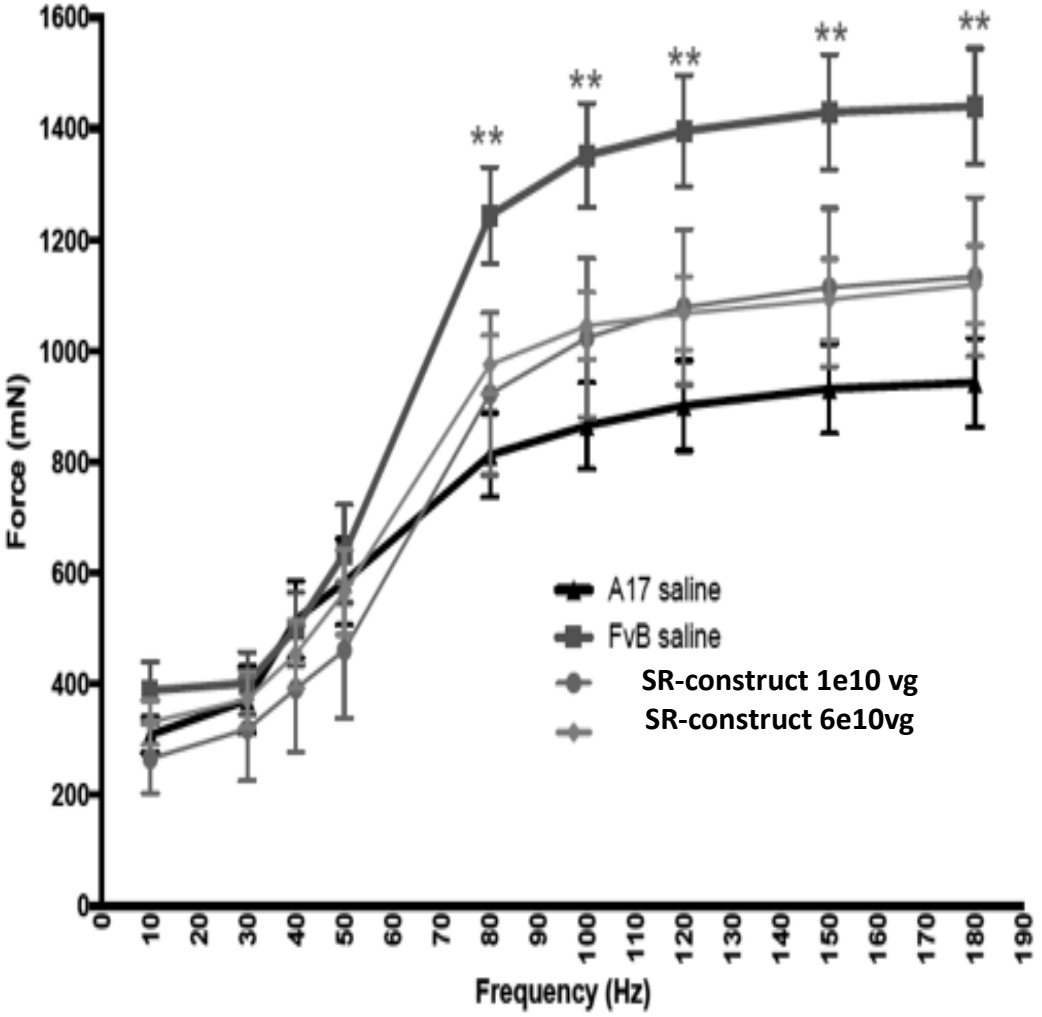


Figure 6A

20 weeks post SR-construct dosing

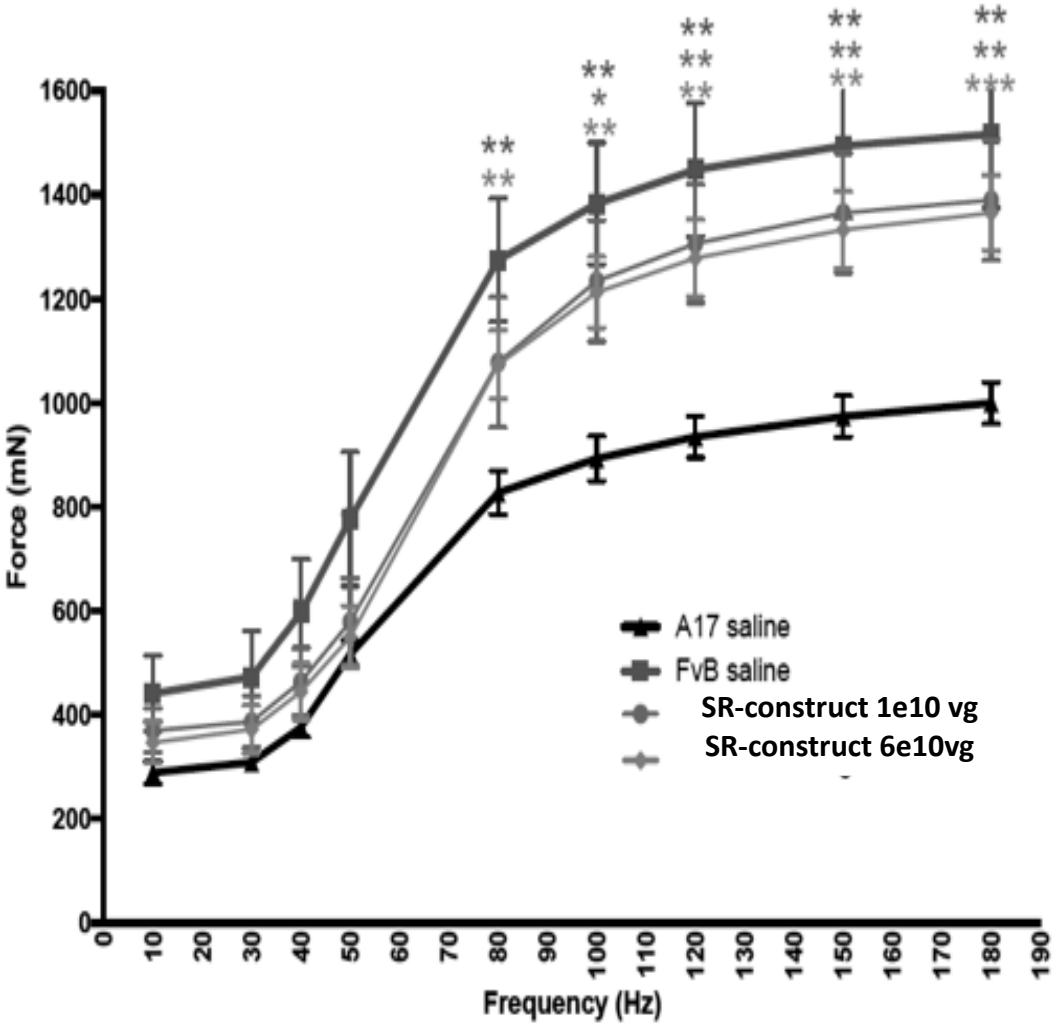


Figure 6B

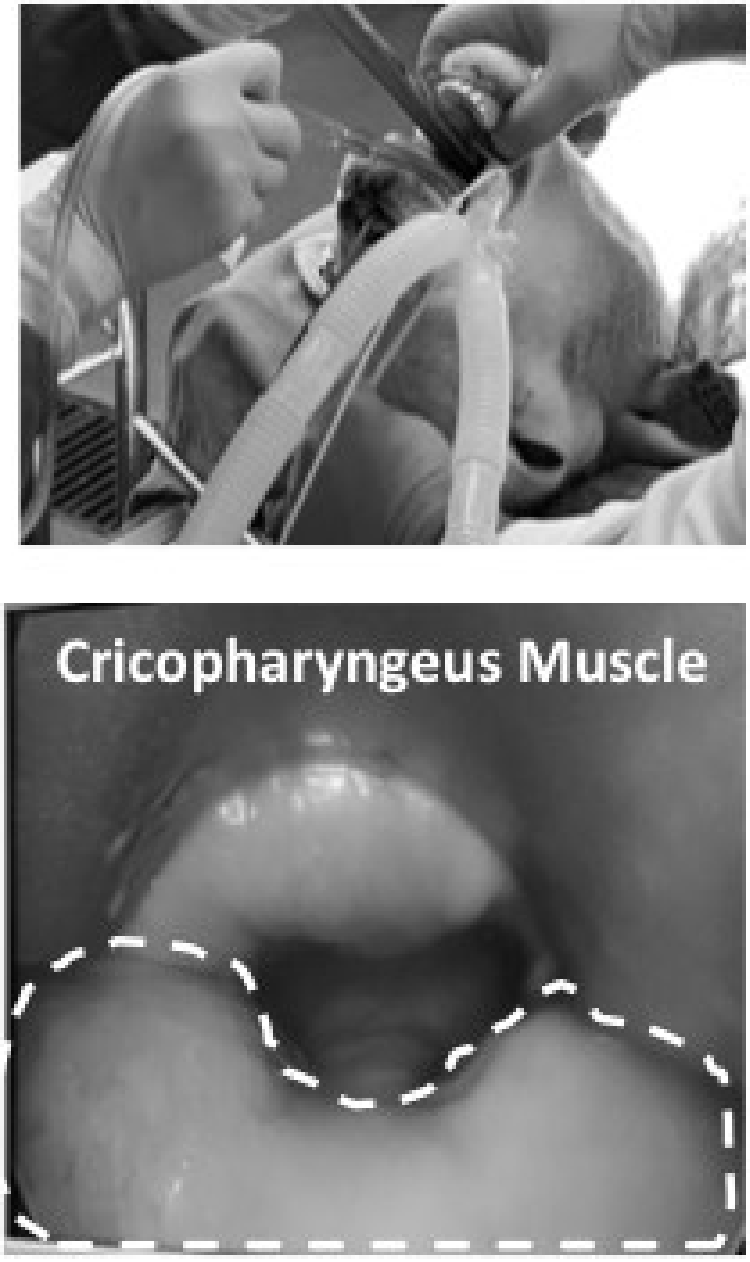


Figure 7A

Pharyngeal Muscles
Esophagus: To Stomach
6
Trachea: To Lungs
3
6
2
5
1
4

Figure 7B

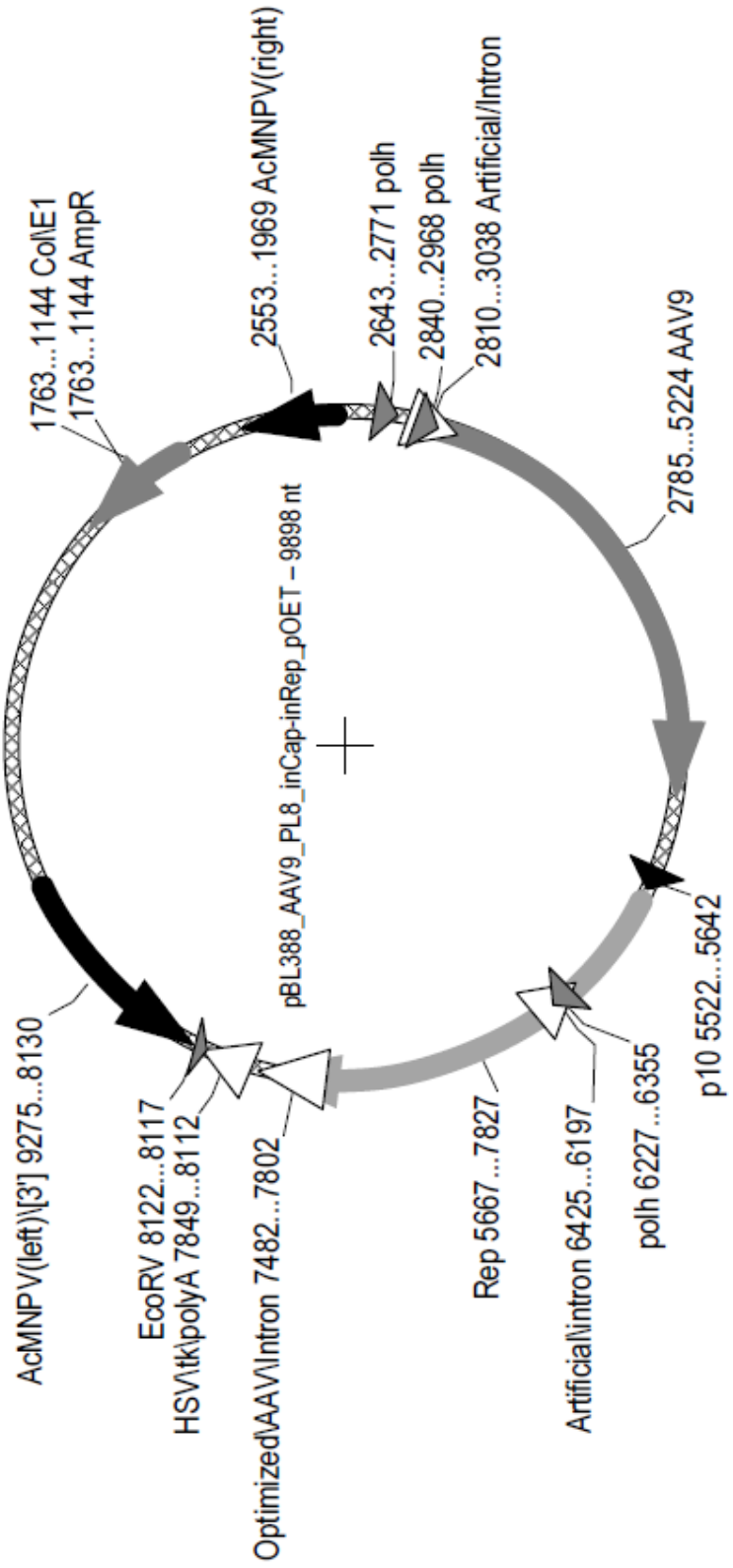

pBL388_AAV9_PL8_inCap-inRep_pOET – 9898 nt
1763...1144 Col\E1
1763...1144 AmpR
2553...1969 AcMNPV(right)
2643...2771 polh
2840...2968 polh
2810...3038 Artificial/Intron
2785...5224 AAV9
p10 5522...5642
polh 6227...6355
Artificial\intron 6425...6197
Rep 5667...7827
Optimized\AAV\Intron 7482...7802
EcoRV 8122...8117
HSV\tk\polyA 7849...8112
AcMNPV(left)\[3'] 9275...8130

Figure 8

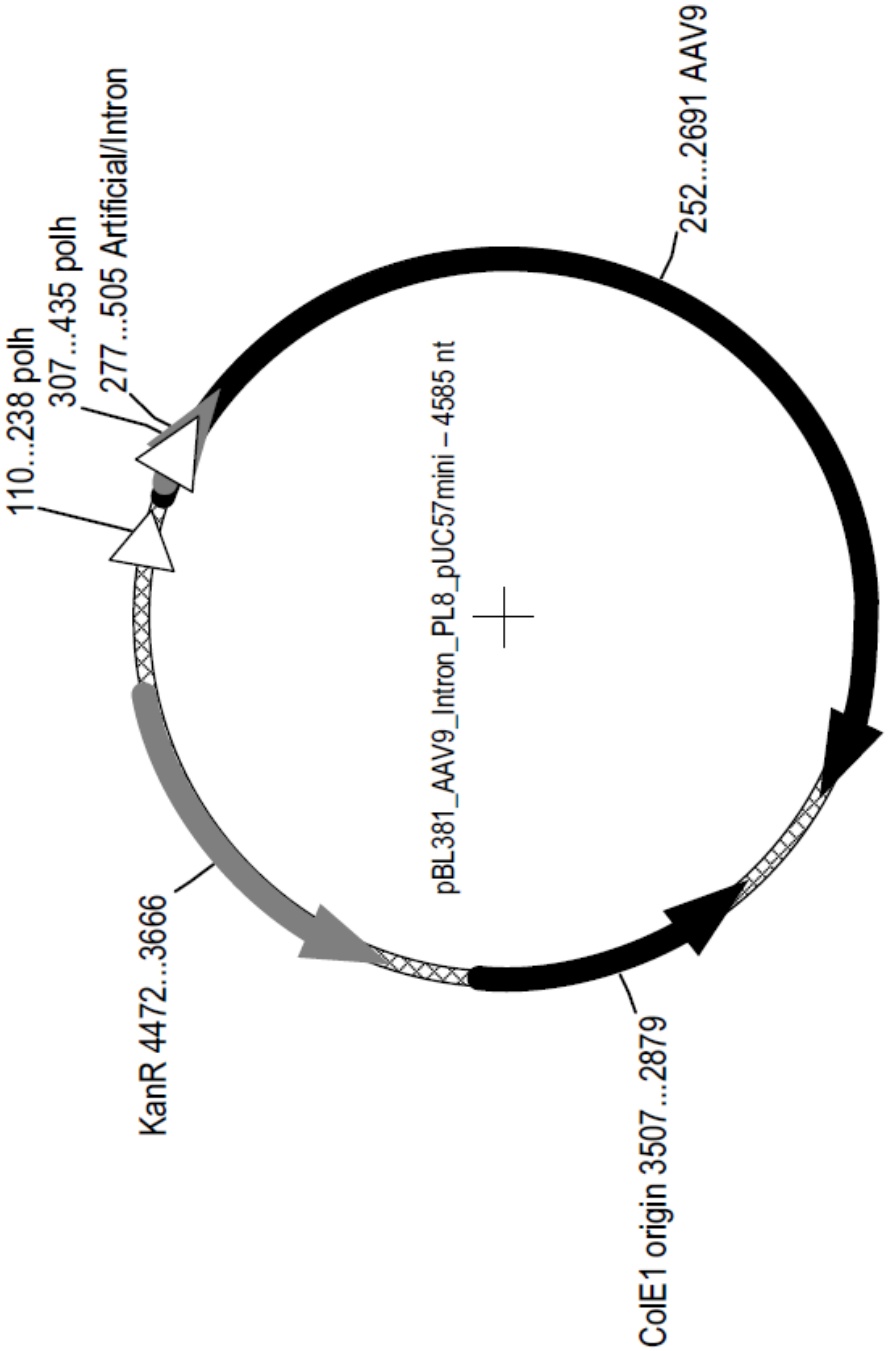

110...238 polh
307...435 polh
277...505 Artificial/Intron
252...2691 AAV9
pBL381_AAV9_Intron_PL8_pUC57mini – 4585 nt
KanR 4472...3666
ColE1 origin 3507...2879

Figure 9

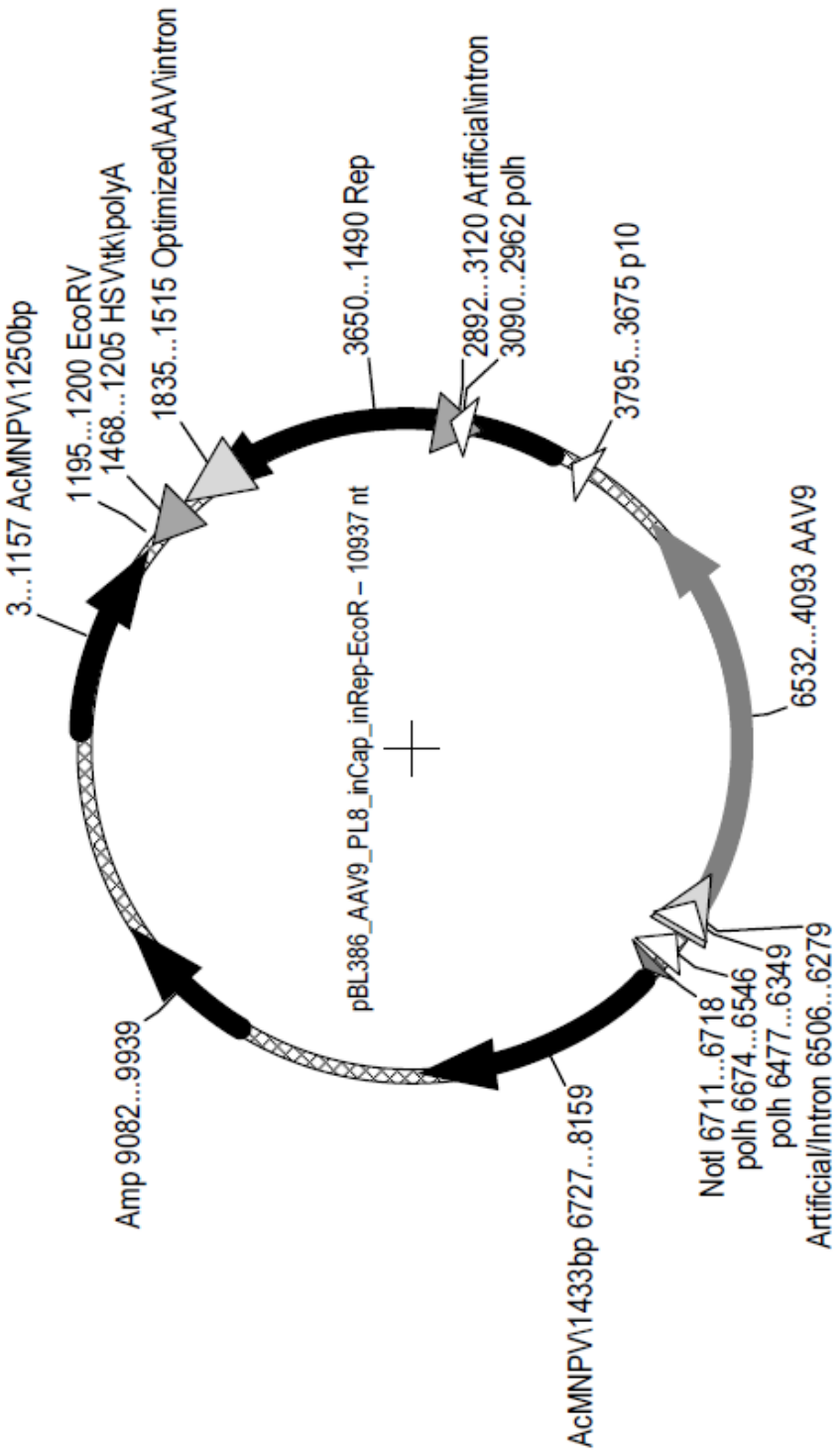

pBL386_AAV9_PL8_inCap_inRep-EcoR – 10937 nt
3...1157 AcMNPV\1250bp
1195...1200 EcoRV
1468...1205 HSV\tk\polyA
1835...1515 Optimized\AAV\intron
3650...1490 Rep
2892...3120 Artificial\intron
3090...2962 polh
3795...3675 p10
6532...4093 AAV9
NotI 6711...6718
polh 6674...6546
polh 6477...6349
Artificial/Intron 6506...6279
AcMNPV\1433bp 6727...8159
Amp 9082...9939

Figure 10

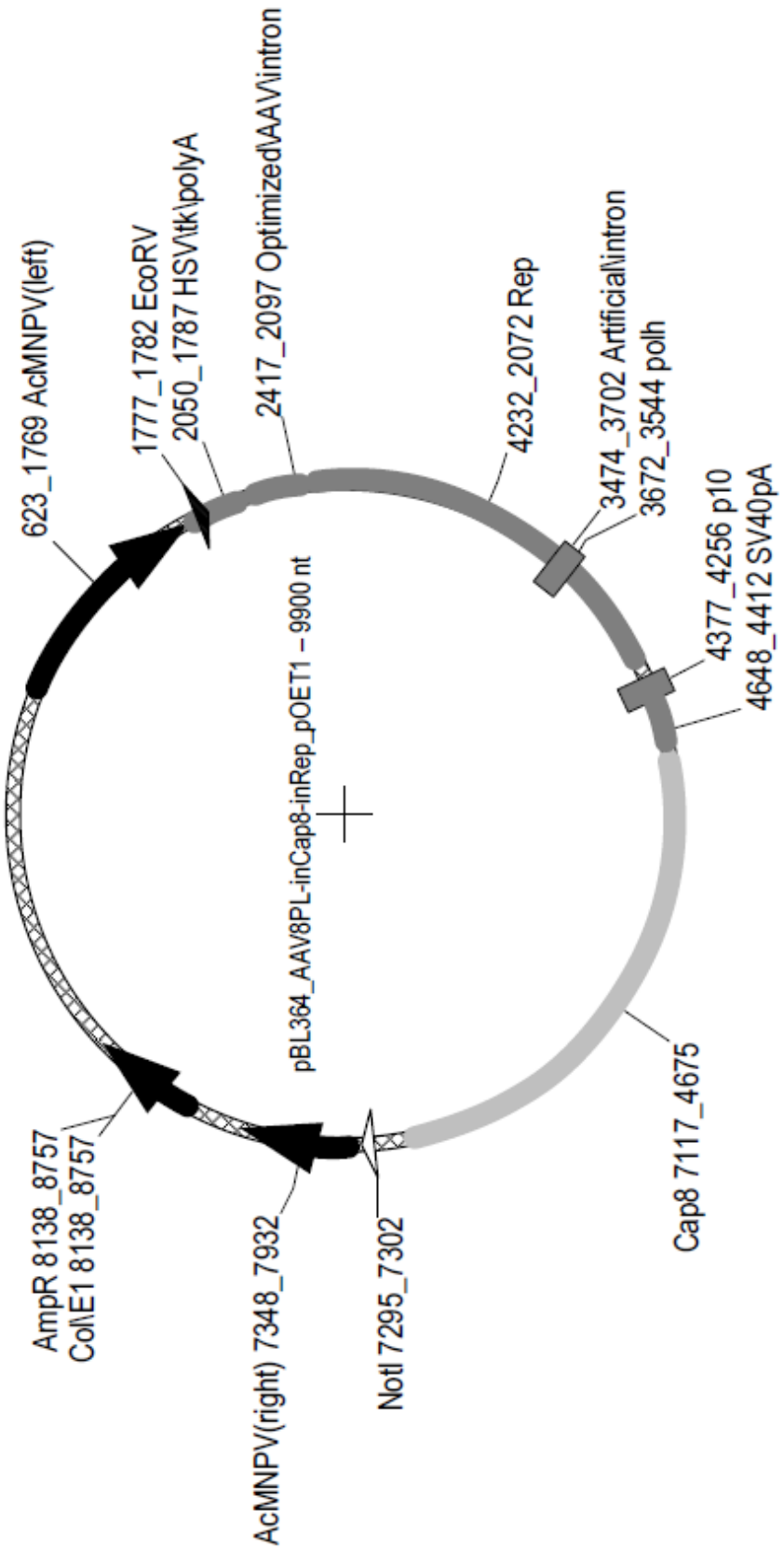

623_1769 AcMNPV(left)
1777_1782 EcoRV
2050_1787 HSV\tk\polyA
2417_2097 OptimizedAAV\intron
4232_2072 Rep
3474_3702 Artificial\intron
3672_3544 polh
4377_4256 p10
4648_4412 SV40pA
pBL364_AAV8PL-inCap8-inRep_pOET1 – 9900 nt
Cap8 7117_4675
NotI 7295_7302
AcMNPV(right) 7348_7932
AmpR 8138_8757
Col\E1 8138_8757

Figure 11

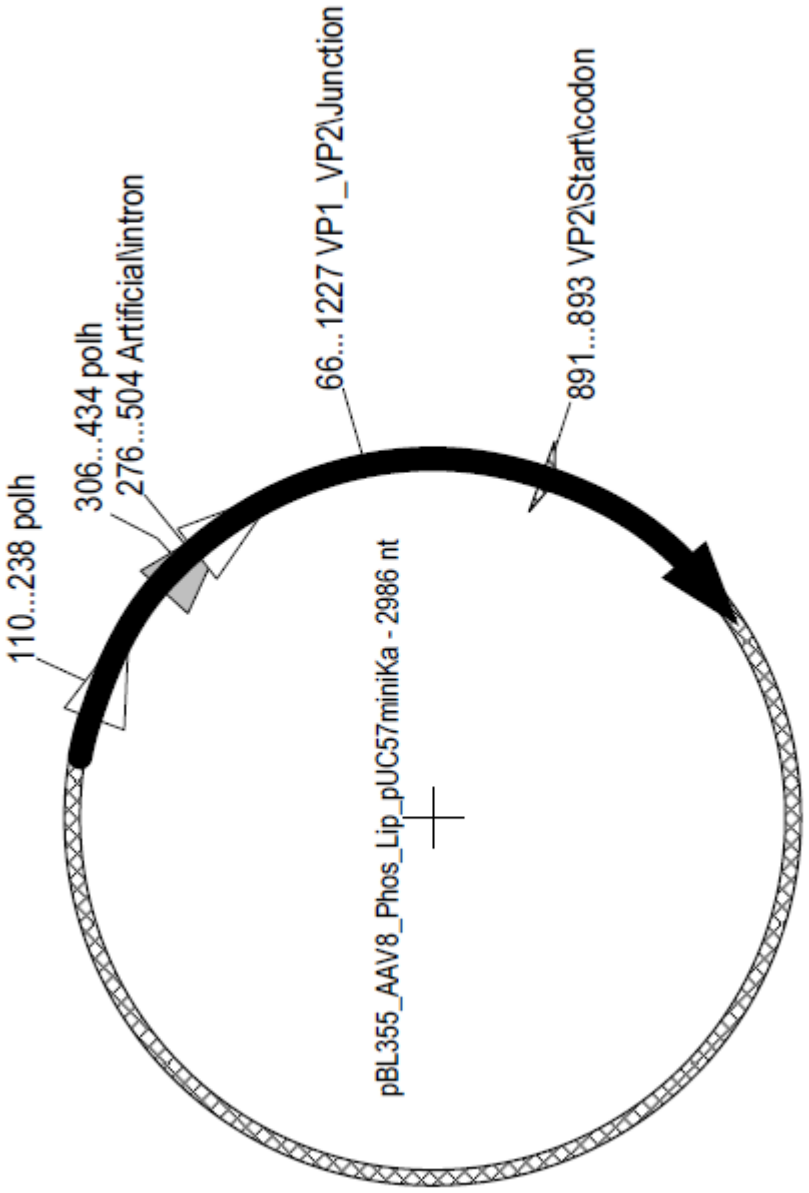

110...238 polh
306...434 polh
276...504 Artificial\intron
66...1227 VP1_VP2\Junction
891...893 VP2\Start\codon
pBL355_AAV8_Phos_Lip_pUC57miniKa - 2986 nt

Figure 12

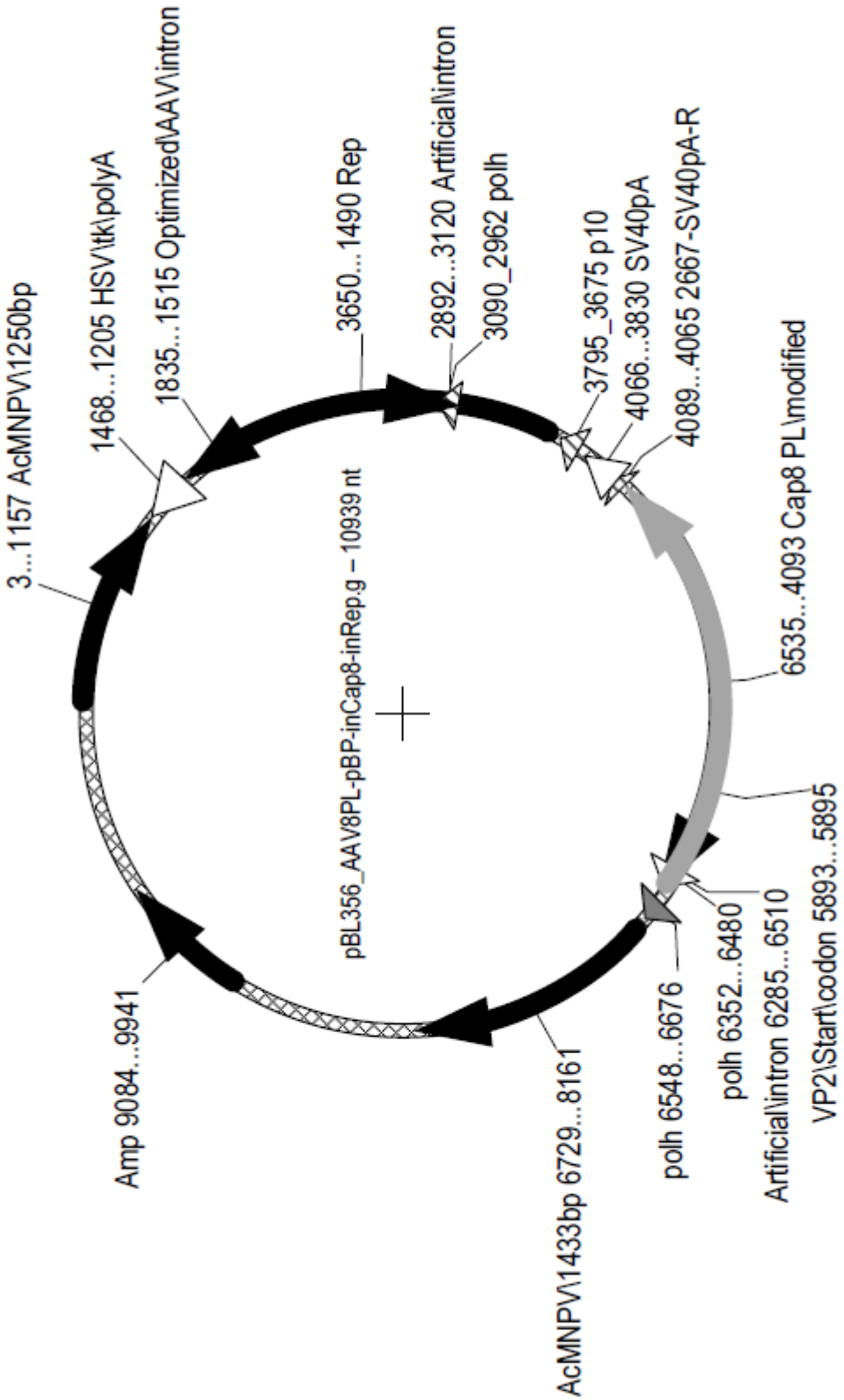

pBL356_AAV8PL-pBP-inCap8-inRep.g – 10939 nt
3...1157 AcMNPV\1250bp
1468...1205 HSV\tk\polyA
1835...1515 OptimizedAAV\intron
3650...1490 Rep
2892...3120 Artificial\intron
3090_2962 polh
3795_3675 p10
4066...3830 SV40pA
4089...4065 2667-SV40pA-R
6535...4093 Cap8 PL\modified
VP2\Start\codon 5893...5895
Artificial\intron 6285...6510
polh 6352...6480
polh 6548...6676
AcMNPV\1433bp 6729...8161
Amp 9084...9941

Figure 13

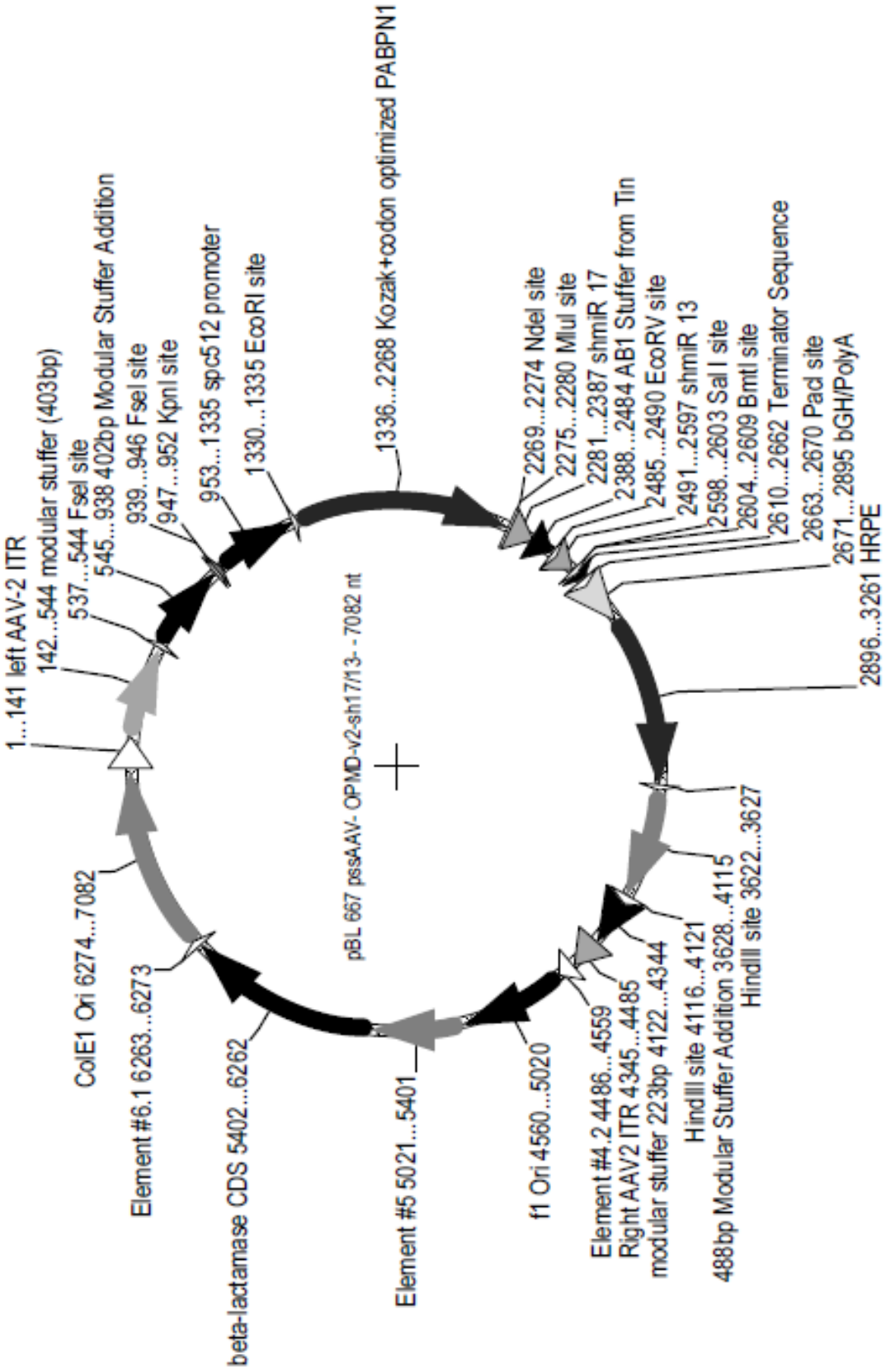

pBL 667 pssAAV- OPMD-v2-sh17/13- - 7082 nt
1...141 left AAV-2 ITR
142...544 modular stuffer (403bp)
537...544 FseI site
545...938 402bp Modular Stuffer Addition
939...946 FseI site
947...952 KpnI site
953...1335 spc512 promoter
1330...1335 EcoRI site
1336...2268 Kozak+codon optimized PABPN1
2269...2274 NdeI site
2275...2280 MluI site
2281...2387 shmiR 17
2388...2484 AB1 Stuffer from Tin
2485...2490 EcoRV site
2491...2597 shmiR 13
2598...2603 Sal I site
2604...2609 BmtI site
2610...2662 Terminator Sequence
2663...2670 PacI site
2671...2895 bGH/PolyA
2896...3261 HRPE
Element #4.2 4486...4559
Right AAV2 ITR 4345...4485
modular stuffer 223bp 4122...4344
HindIII site 4116...4121
488bp Modular Stuffer Addition 3628...4115
HindIII site 3622...3627
f1 Ori 4560...5020
Element #5 5021...5401
beta-lactamase CDS 5402...6262
Element #6.1 6263...6273
ColE1 Ori 6274...7082

Figure 14

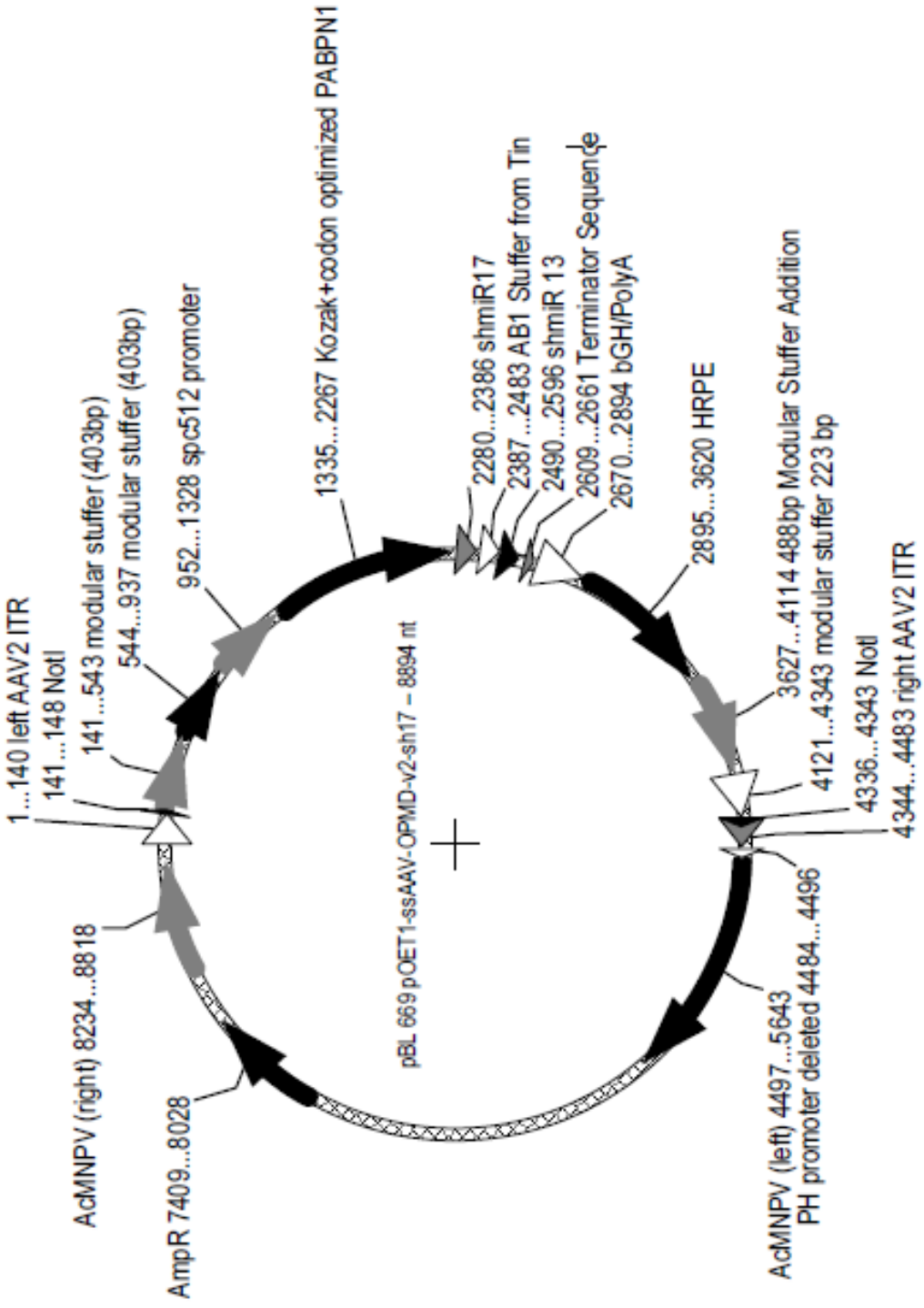
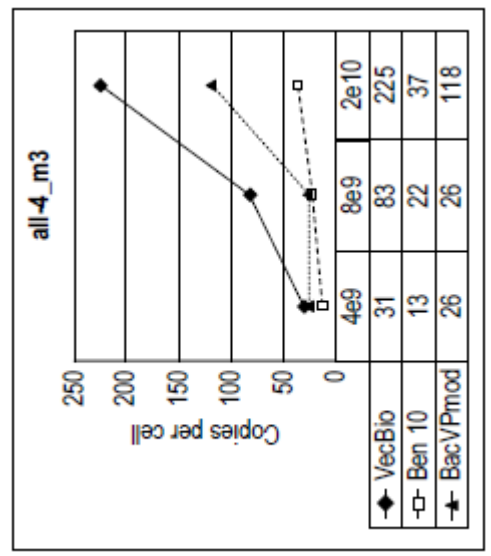
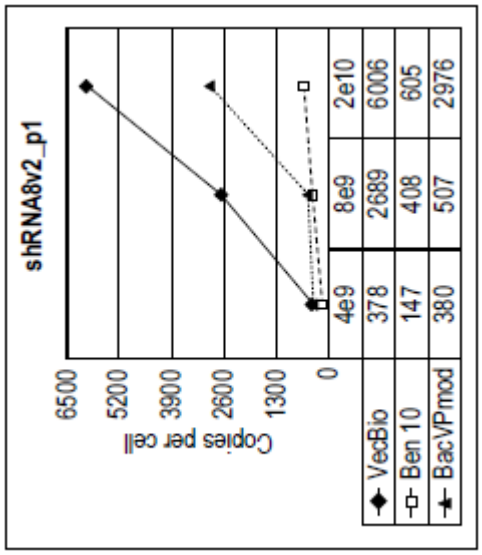

1...140 left AAV2 ITR
141...148 NotI
141...543 modular stuffer (403bp)
544...937 modular stuffer (403bp)
952...1328 spc512 promoter
1335...2267 Kozak+codon optimized PABPN1
2280...2386 shmiR17
2387...2483 AB1 Stuffer from Tin
2490...2596 shmiR 13
2609...2661 Terminator Sequence
2670...2894 bGH/PolyA
2895...3620 HRPE
3627...4114 488bp Modular Stuffer Addition
4121...4343 modular stuffer 223 bp
4336...4343 NotI
4344...4483 right AAV2 ITR
AdMNPV (left) 4497...5643
PH promoter deleted 4484...4496
AmpR 7409...8028
AdMNPV (right) 8234...8818
pBL 669 pOET1-ssAAV-OPMD-v2-sh17 – 8894 nt

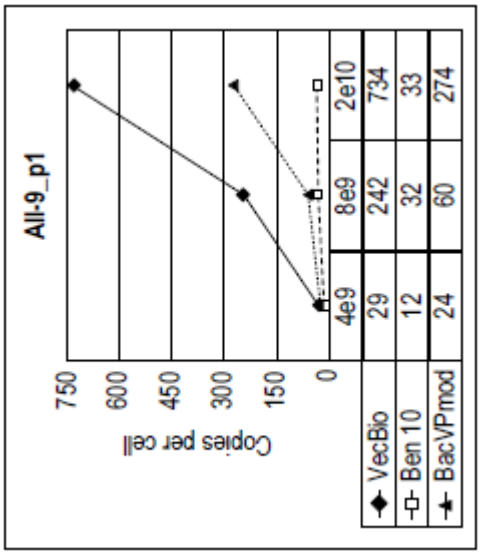
Figure 15



A



B



C

Figure 16

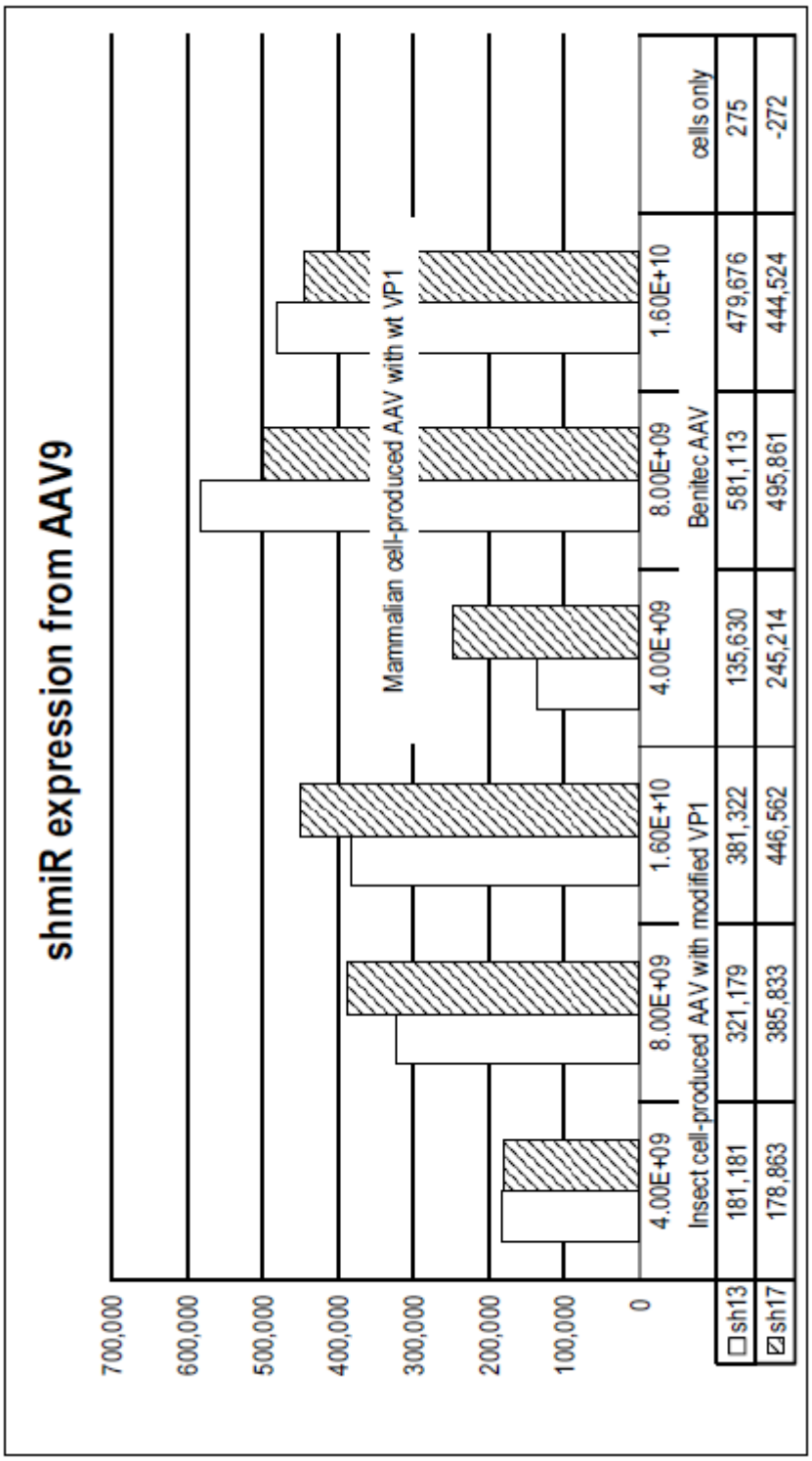

shmiR expression from AAV9
700,000
600,000
500,000
400,000
300,000
200,000
100,000
0
Mammalian cell-produced AAV with wt VP1
Insect cell-produced AAV with modified VP1
Benitec AAV
cells only
4.00E+09
8.00E+09
1.60E+10
4.00E+09
8.00E+09
1.60E+10
sh13
sh17
181,181
178,863
321,179
385,833
381,322
446,562
135,630
245,214
581,113
495,861
479,676
444,524
275
-272

Figure 17