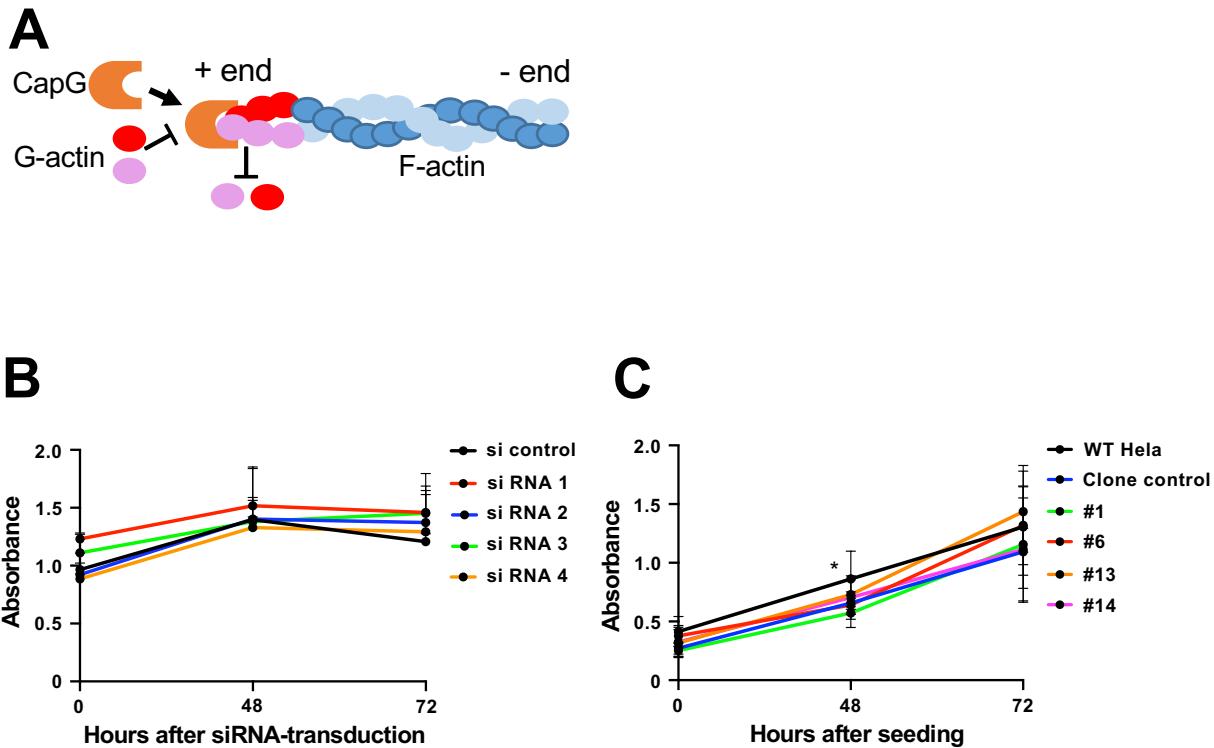
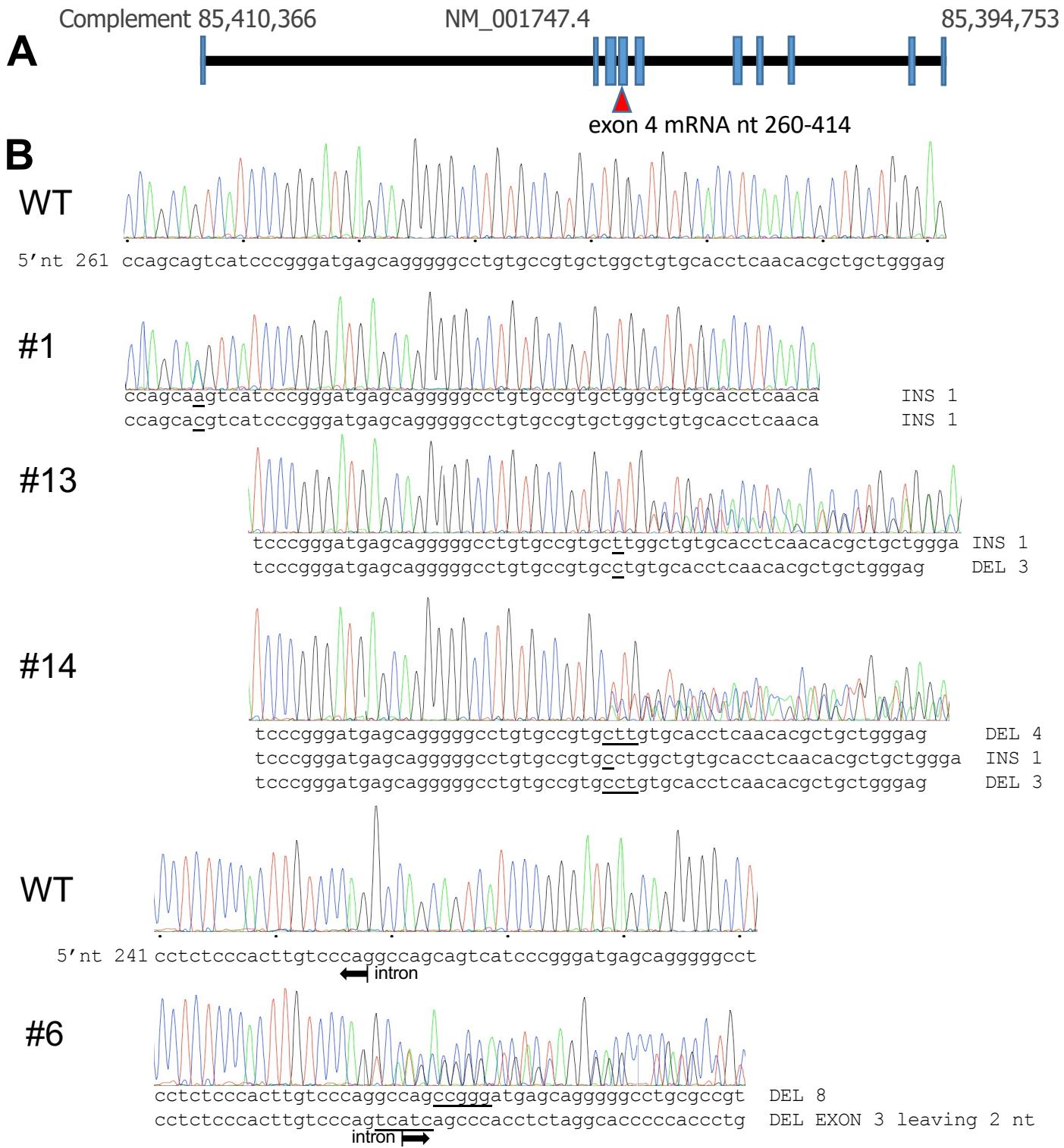


Supplementary Figures and Tables.

Mori et al. CAPG is required for Ebola virus infection.

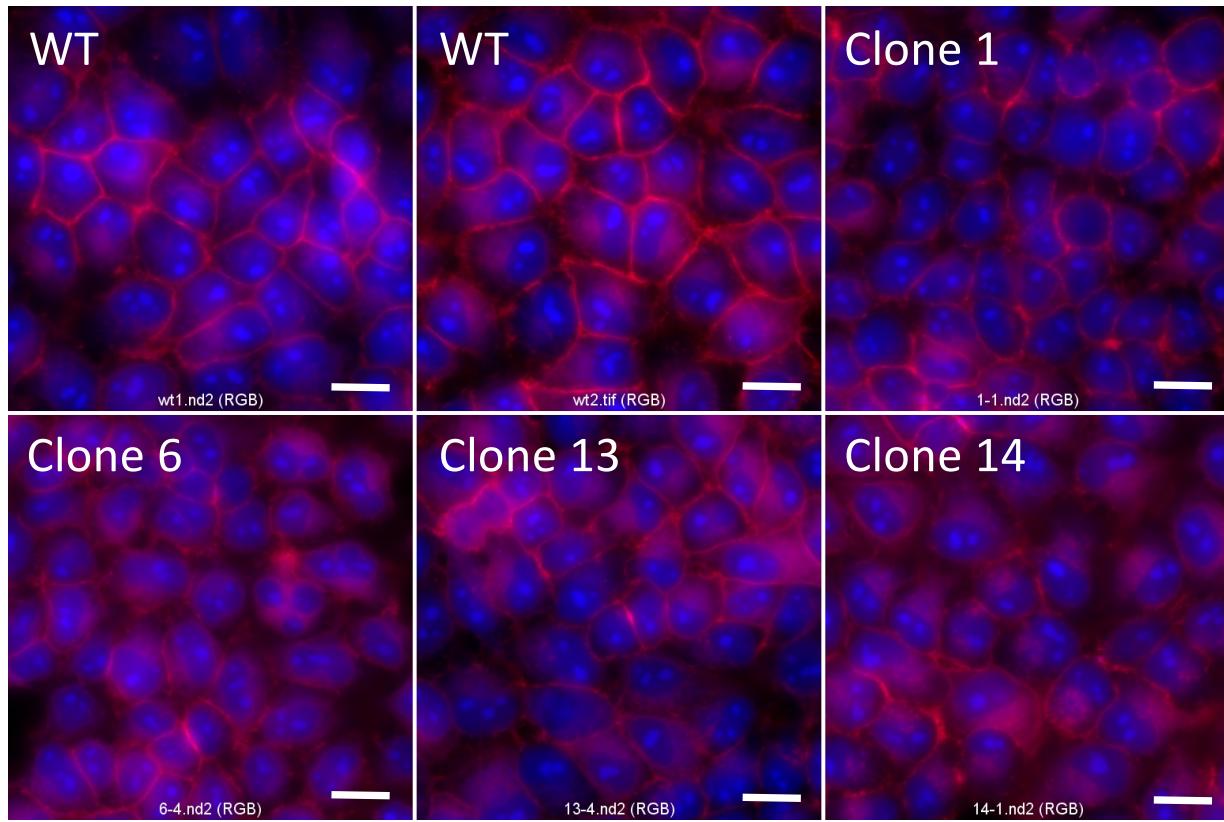


Supplementary Figure S1. Schematic of CAPG function and cell viability after transduction of siRNA and CAPG knockout cells. **A.** CAPG functions to cap the barbed, growing end of actin filaments and actin branches. This prevents addition of new G-actin monomers as well as release of monomers from already formed filaments, stabilizing them. **B.** Cell viability of Hela cells after transduction of siRNA and **C.** knockout (KO) clones in a time-course of post-transfection or seeding, respectively. An MTT assay was used to check viability and read using absorbance of samples seeded on a 96 well plate. Absorbance was measured at 575 nm, and 675 nm wavelengths used for background detection. Statistical difference was calculated by One-way ANOVA with Tukey's multiple comparisons test at each time point. *, P < 0.05.

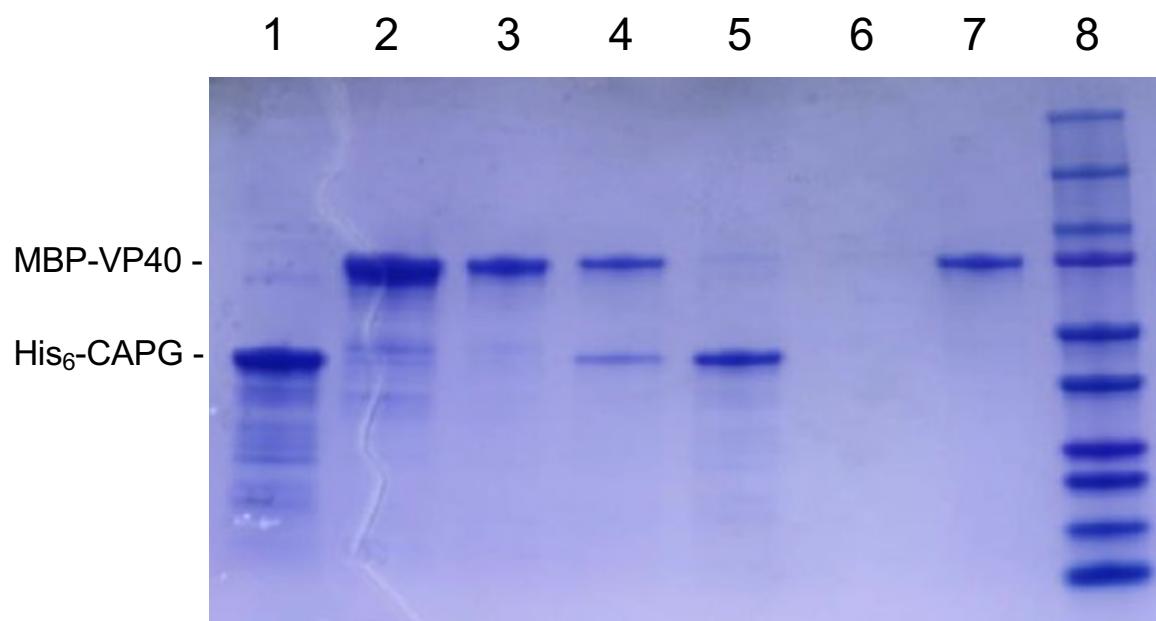


Supplementary Figure S2. Sequence information for INDELS of CAPG KO clones.

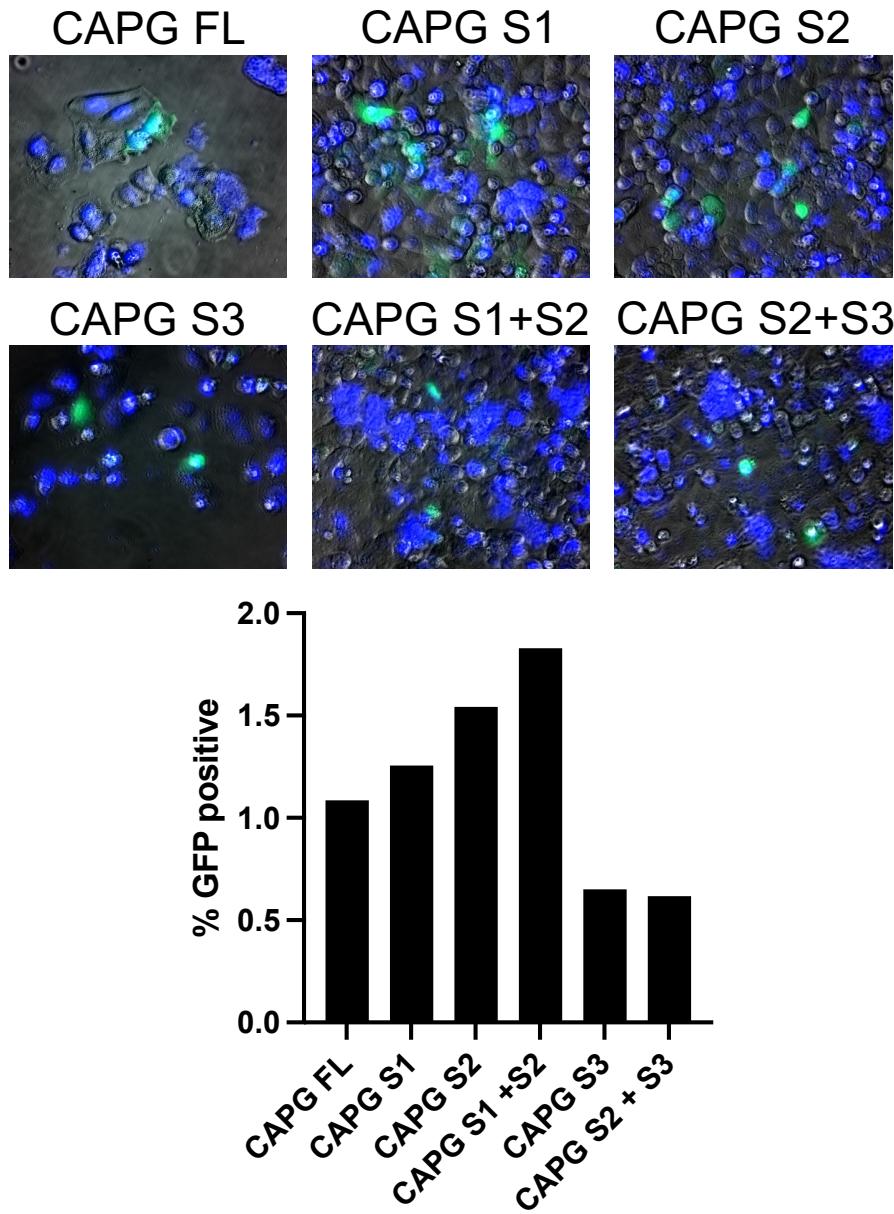
A. Gene structure of CAPG modified from NCBI using CAPG variant 1 NM_001747.4 with exons indicated by rectangles. Chromosomal position information is shown at top and exon 4 (spanning mRNA nt 260-414) targeted by CRISPR indicated by arrowhead. **B.** Sanger sequencing for regions targeted by CRISPR. Below the wild type (WT) chromatogram are the nucleotide number and sequence. Below the WT are aligned the chromatograms of each clone together with the inferred sequences for each allele and defect indicated at right. INS: insertion, DEL: deletion. Underlined sequence indicates area of change when compared to wild type sequences. Clone 6 had deletion of 8 nt for one allele and was unusual with deletion of most of exon 4 in a second allele, leaving 2 nt. Intron junctions for this region are indicated with arrows.



Supplementary Figure S3. Phalloidin staining of KO and KD cell clones. The indicated clonal cell lines described in Fig. S2, were fixed in formalin and stained with phalloidin to detect F-actin (red) and Hoechst 33342 to detect cell nuclei (blue). Scale bar is 20 μ m.



Supplementary Figure S4. In vitro pulldown assay does not show VP40 binding to CAPG. MBP-tagged VP40 was immobilized onto amylose resin prior to incubation with His₆-CAPG. Lanes are: 1, His₆-CAPG; 2, MBP-eVP40; 3, MBP-VP40 bound beads; 4, MBP-VP40 beads with His₆-CAPG; 5, flow through; 6, final wash; 7, final bound beads; 8, marker.



Supplementary Figure S5. Measurement of split GFP construct expression. Cells were co-transfected with plasmids encoding each indicated construct together with the GFP1-10 (lacking only the B11 peptide). The resulting trans-complementation, which occurs spontaneously, was used as an indicator of protein expression efficiency. The number of cells expressing GFP was calculated from images taken on an Opera Phenix automated microscope. Example images are shown at top with quantification shown below for one experiment.

Supplemental tables.

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	Sequence (5' to 3')	Exon
siRNA 1	CTGGTGTGGTGGAAAGTCCAA	6
siRNA 2	CAGGTGGAGATTGTCAGT	6
siRNA 3	CGGGCTCTGTGGCAAGATCTA	8
siRNA 4	AAGGAGGGCAACCTGAGGAA	7

Supplementary Table S1. Sequences of siRNA used to target CAPG mRNA.

siRNAs were synthesized and ordered from Qiagen. siRNA 1 and 4 were custom designed and siRNA 2 and 3 were from FlexiTUBE siRNA (#1027417). Control siRNA was AllStars Neg. Control siRNA, (Qiagen, # 1027281). Exons of CAPG targeted by each are indicated.

Host	Gene target	Primer/Probe	Amplicon size (bp)	Sequence (5'-3')
Ebola virus	NP	Forward	80	GCAGAGCAAGGACTGATACA
		Reverse		GTTCGCATCAAACGGAAAAT
		Probe		FAM-CAACAGCTT-ZEN-GGCAATCAGTAGGACA-IABkFQ
Human	GAPDH	Forward	143	ACATCGCTCAGACACCATG
		Reverse		GTAGTGAGGTCAATGAAGGG
		Probe		Cy5-AAGGTCGGAGTCAACGGATTGGTC-IAbRQSp

Supplementary Table S2. Primers and probe sets used for detecting EBOV viral RNA by RT-qPCR.

Primers and probes were constructed for targeting mRNA of each gene based on the indicated references and purchased from Integrated DNA Technologies (IDT) after synthesizing them. Human GAPDH was used as a housekeeping gene. Both reporter dye and quencher were attached to 5' and 3' of probes, respectively. A ZEN quencher was added in the middle of probes as indicated. ZEBOV = Zaire Ebola virus.

Supplementary Table S3. Listing of oligonucleotide sequences used for cloning and sequencing of constructs.

PLASMID CONSTRUCT	FP	FORWARD PRIMER SEQ	CP	CORE PRIMER SEQ	RP	REVERSE PRIMER SEQ
pCDNA5-FRT-TO-EBOV-VP30-CT-GFP-B10	DJL3600	TGAGGGTACCCCTGGAGGCCGATCCGGCGGAGGTAGCATGGACCTGCTGACGAC	DJL3616	ATGGACCTGCCGTACGCCACTACCTGTCACCCAGACCATCCTGTCAGGACCTGAAAC	DJL3611	TTAAGGTACCGGGCCCCCTCGAGTCAGTTCAAGGCCTTGACAGG
pCDNA5-FRT-TO-NT-GFP10-EBOV-VP40	DJL3769	GGACTTAGCGTTAACCTTAAGCTTGCCACCATGGACTCTGCTGACGACCA	DJL3616	ATGGACCTGCCGTACGCCACTACCTGTCACCCAGACCATCCTGTCAGGACCTGAAAC	DJL3770	GGCAATAAACCCGCTCATGGATCCACGGCCACTGCGCTTCAAGGCTTGGACAGG
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pCAPG-CT-GFP-B11	DJL3808	AGTGAAACCGTCAGATCCGCTAGGCCATGATCACAGCAATTCCACAG			DJL3813	TGATTATGATAGTAGCTGGGGCCGCTATGATGATCGTAAATGCC
pCDNA5-FRT-TO-NT-GFP-B11-CAPG-S1	DJL3810	GCTGGATTACCGATGATCAGGGAGGGTCCATGACACAGCAATTCCACAG			DJL3861	GGCCCTCTAGACTCGAGCTCACCTCCACTCTCTCT
pCDNA5-FRT-TO-NT-GFP-B11-CAPG-S2	DJL3863	GCTGGATTACCGATGATCAGGGAGGGTCCATGAAAGCATAAGAC			DJL3862	GGCCCTCTAGACTCGAGCTCACGGGCTGGCTTGAC
pCAPG-S3-CT-GFP-B11	DJL3864	AGTGAAACCGTCAGATCCGCTAGGCCATGAAAGAGGAAATCGAGGGAGGA			DJL3864	CTTTGCTACCTCCGCTGATCTCCCAATCTTGAAAGACTGC
pCDNA5-FRT-TO-NT-GFP-B11-CAPG-S1+S2	DJL3810	GCTGGATTACCGATGATCAGGGAGGGTCCATGACACAGCAATTCCACAG			DJL3862	GGCCCTCTAGACTCGAGCTCACGGGCTGGCTTGAC
pCAPG-S2+S3-CT-GFP-B11	DJL3865	AGTGAAACCGTCAGATCCGCTAGGCCATGTTCCATAAGAC			DJL3809	CTTTGCTACCTCCGCTGATCTCCCAATCTTGAAAGACTGC
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pCDNA5-FRT-TO-NT-GFP-B11-UTRN-CH	DJL3992	GCTGGATTACCGATGATCAGGGAGGGTCCATGCCAAAGTGGAGAACATGA			DJL3993	GGCCCTCTAGACTCGAGCGGGCTGATGCTGGAGGTAG
pPABP1-CT-GFP-B11	DJL3612	CCACCGGTGTTCAACTGTGATCCAGGGGGAGTAGCGAAAGCGAGACCATATGG	DJL3617	AAAGCGAGACCATATGGTTGCTGAGTATGTTACGGCGCTGGCATTACCGATGCATC	DJL3613	TGATTATGATAGTAGCTGGGGCCGCTATGATGATCGTAAATGCC

Notes:

GFP-B10 is the same as GFP10

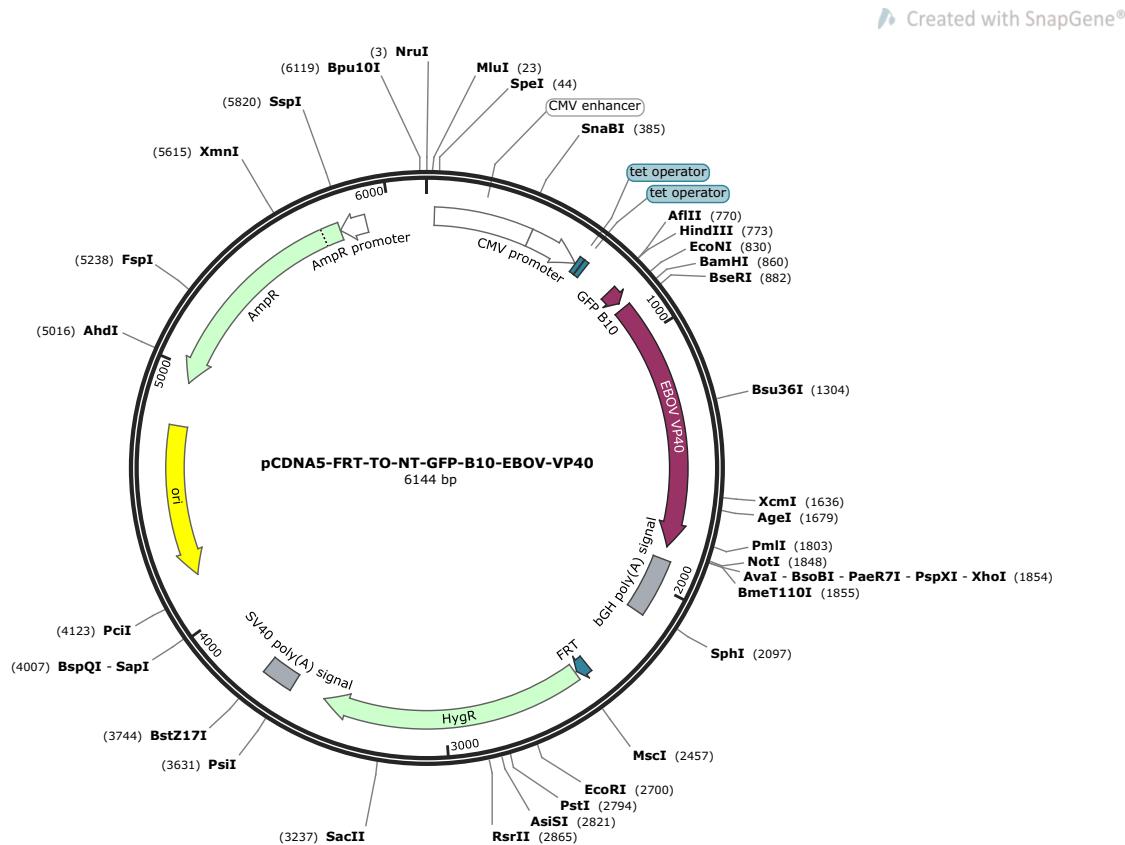
GFP-B11 is the same as GFP11

NT = N-terminal fusion of tag

CT = C-terminal fusion of tag

Supplementary Table S4. Plasmid maps of constructs.

Mori et al Supplemental Materials
Plasmid maps and sequences



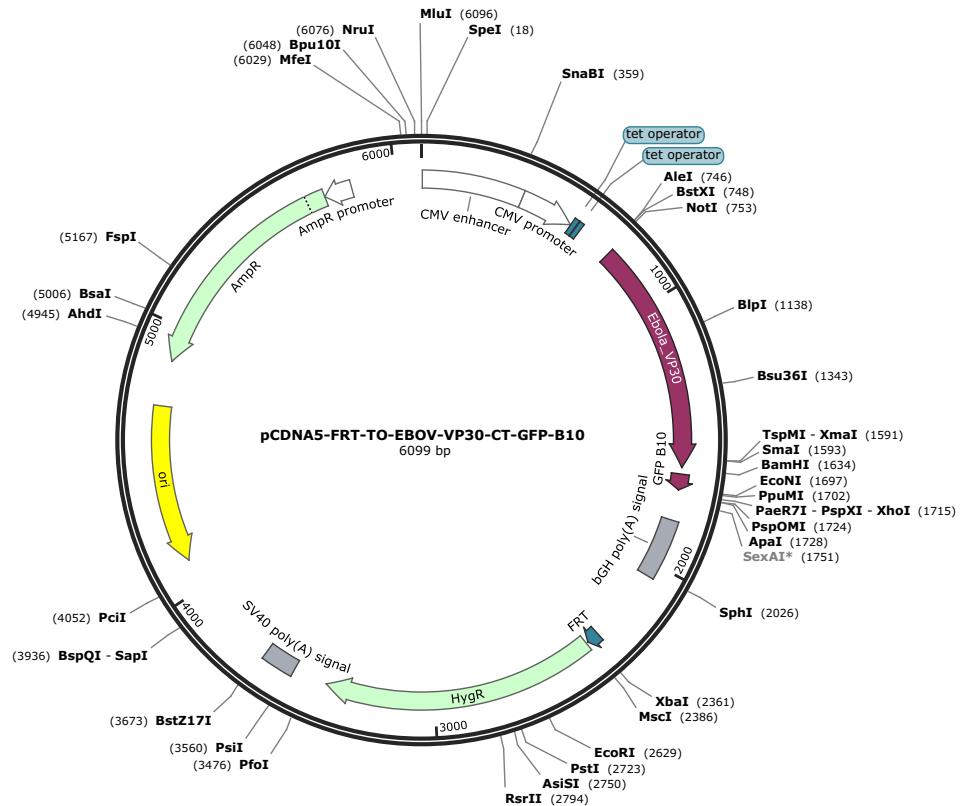
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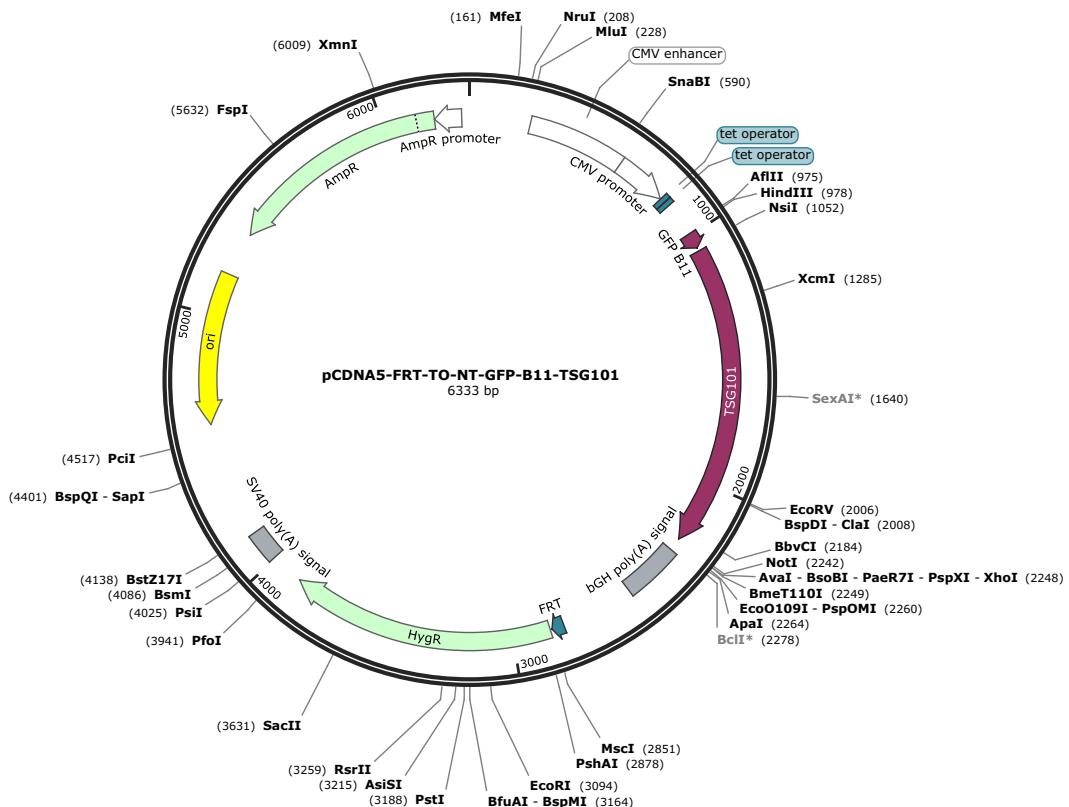
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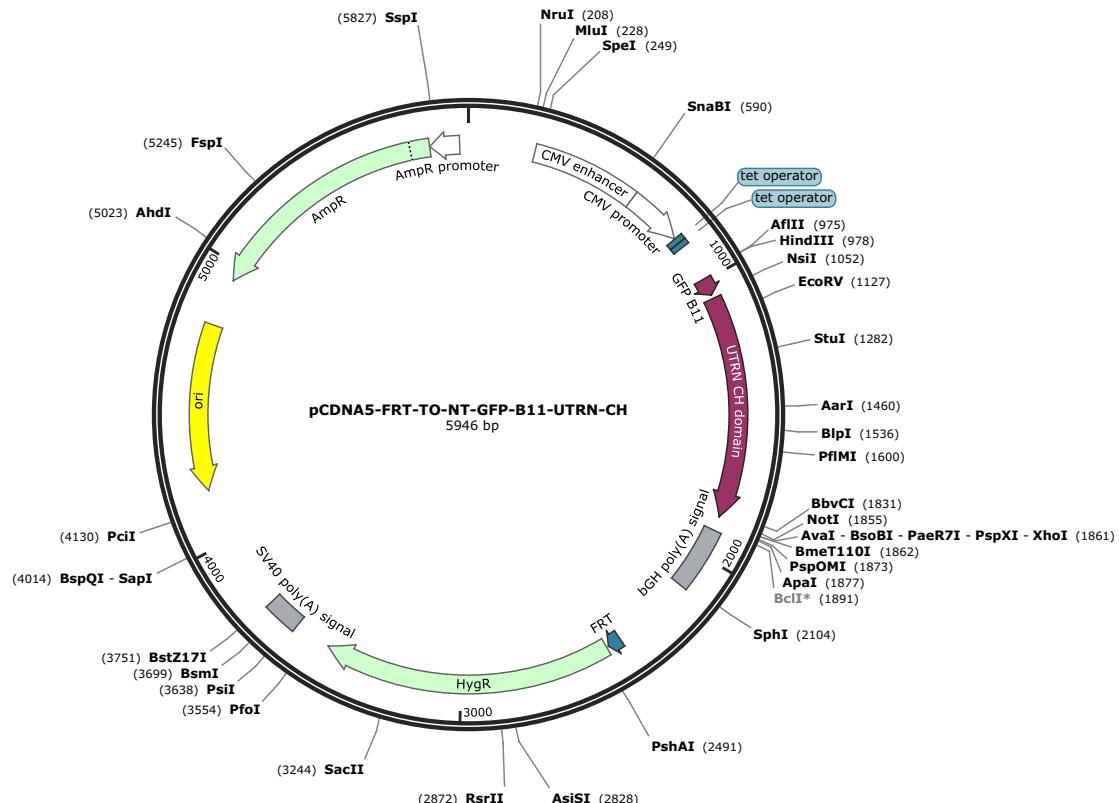
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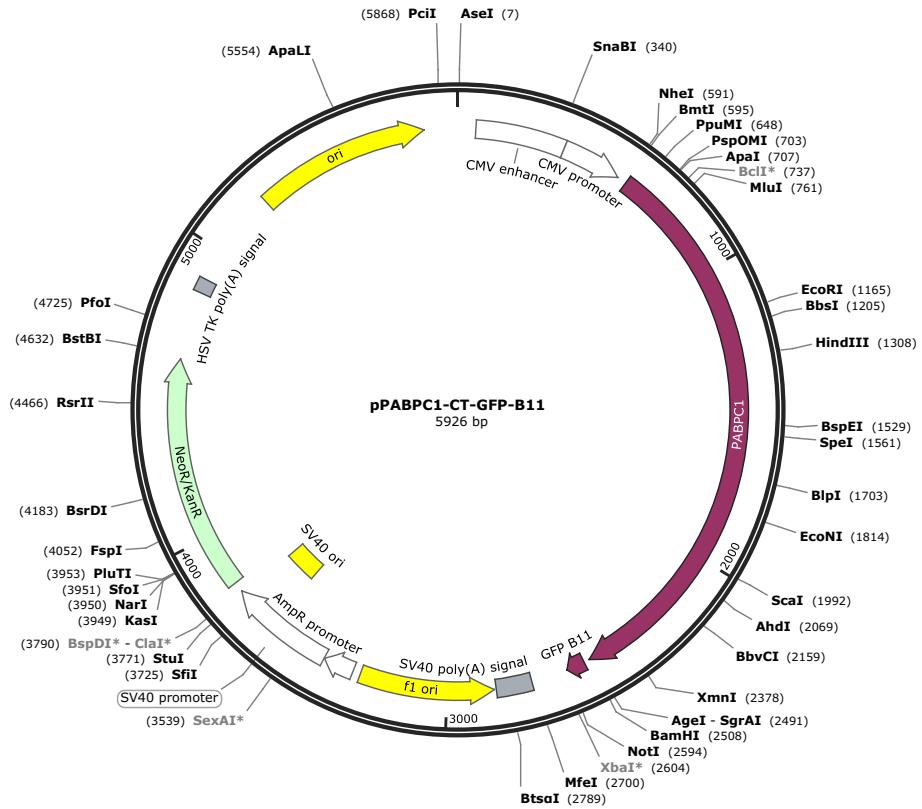
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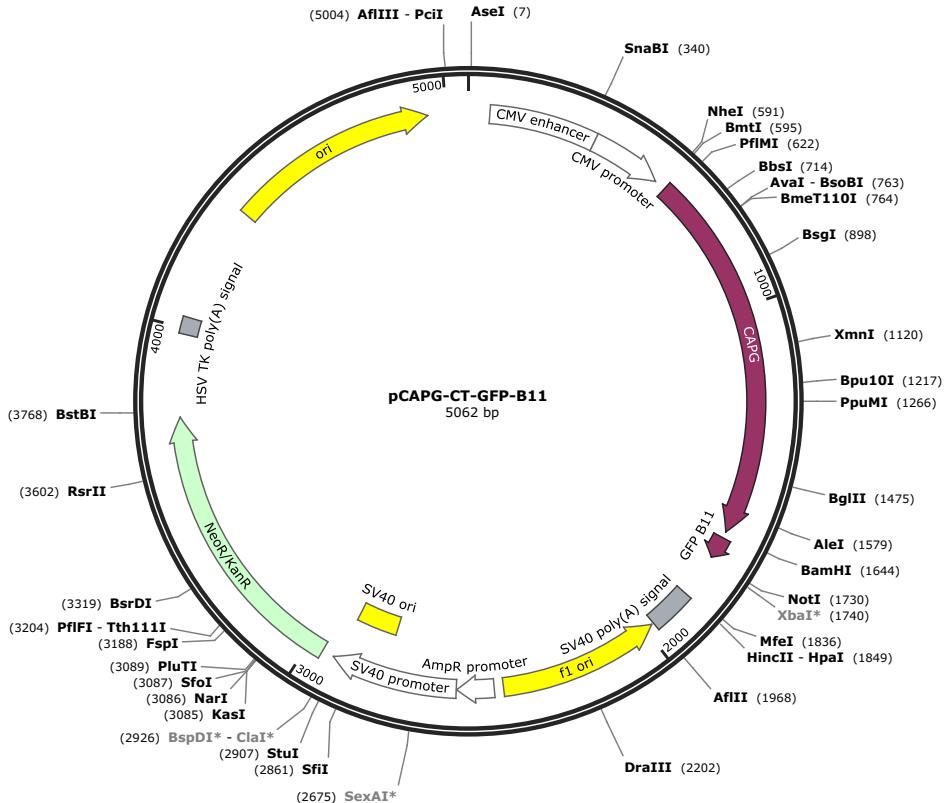
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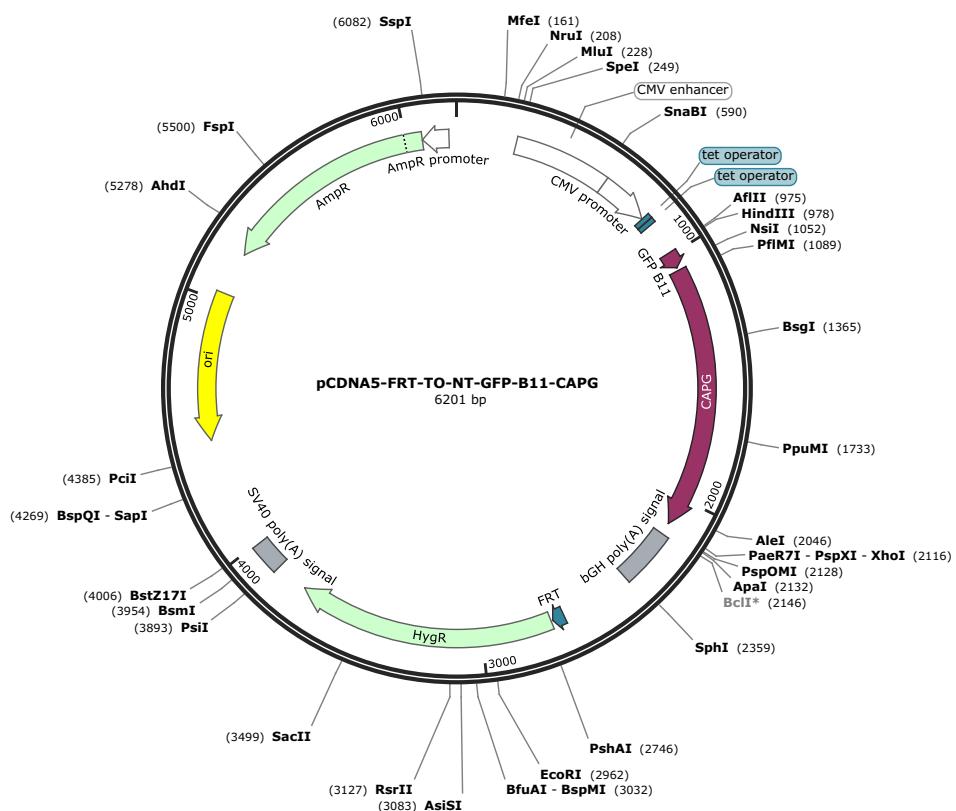
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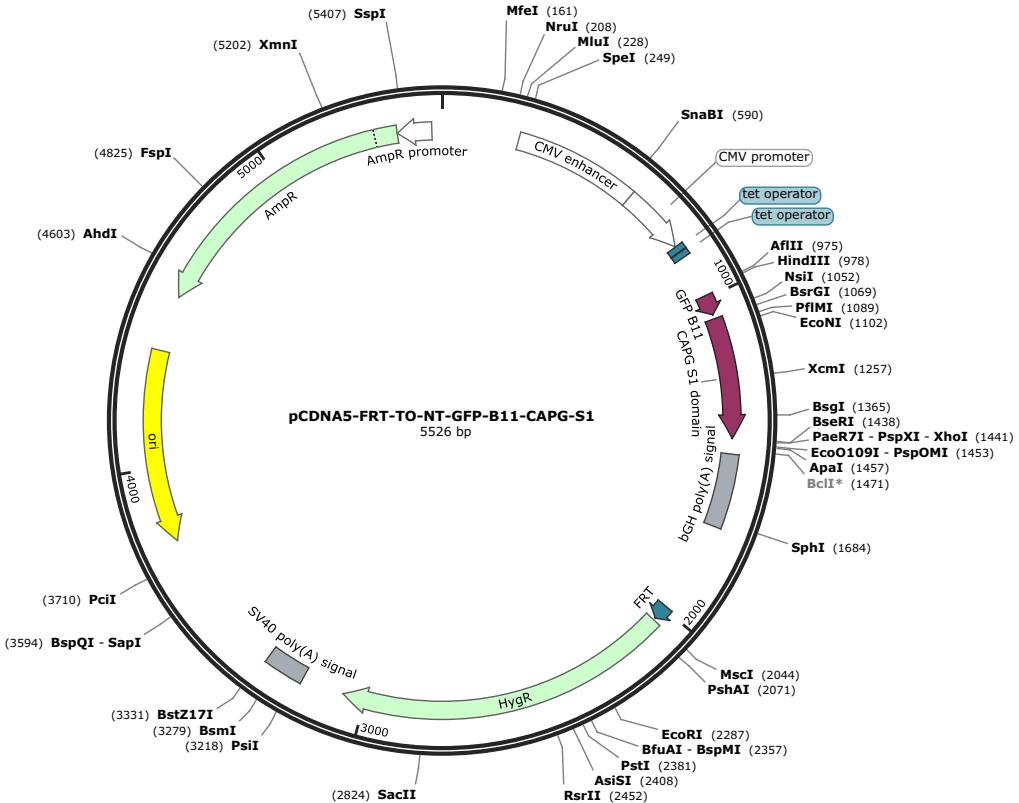
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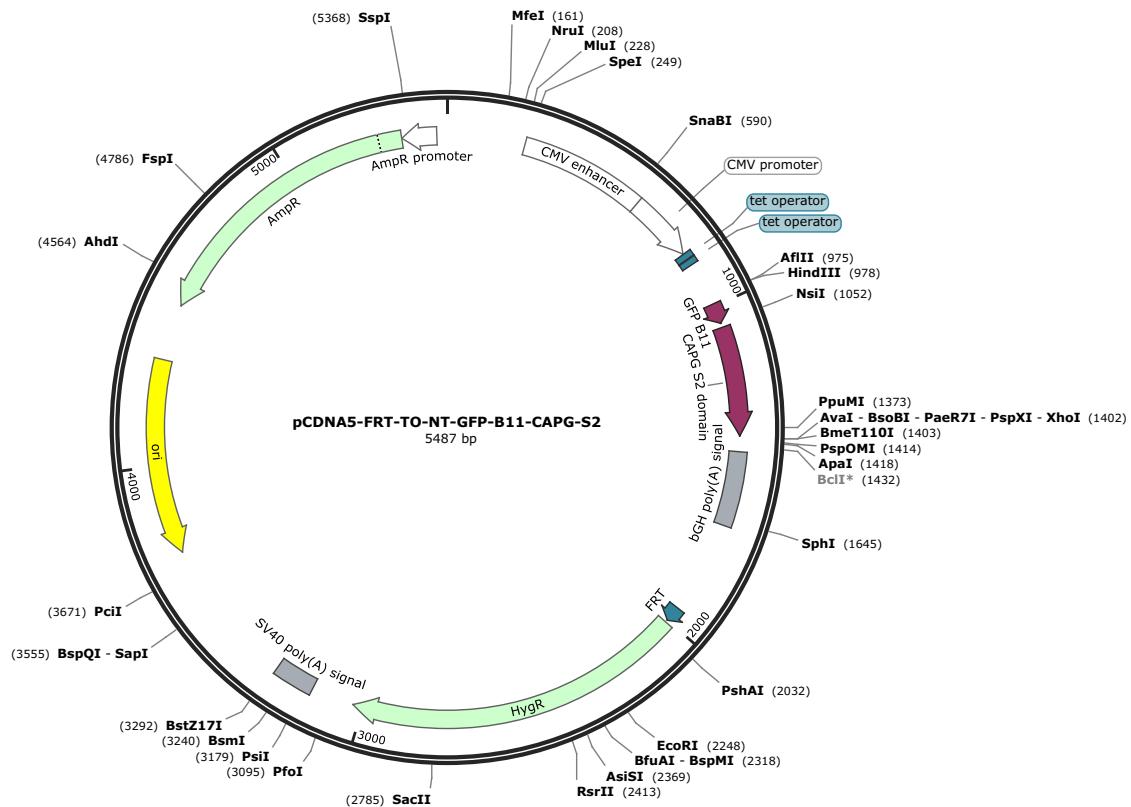
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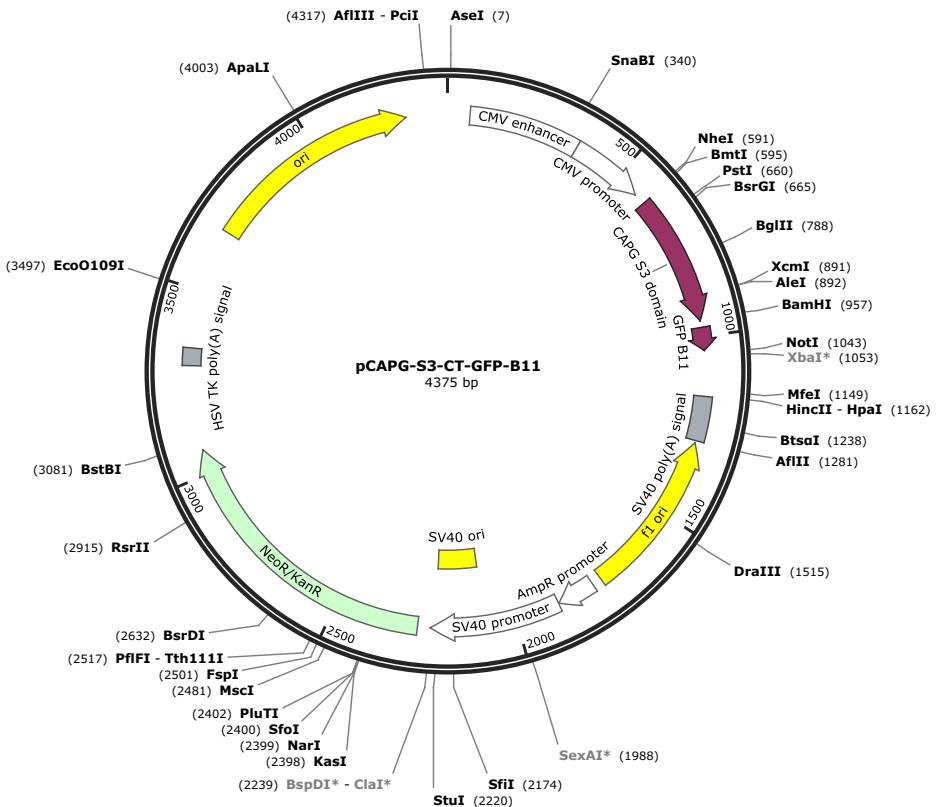
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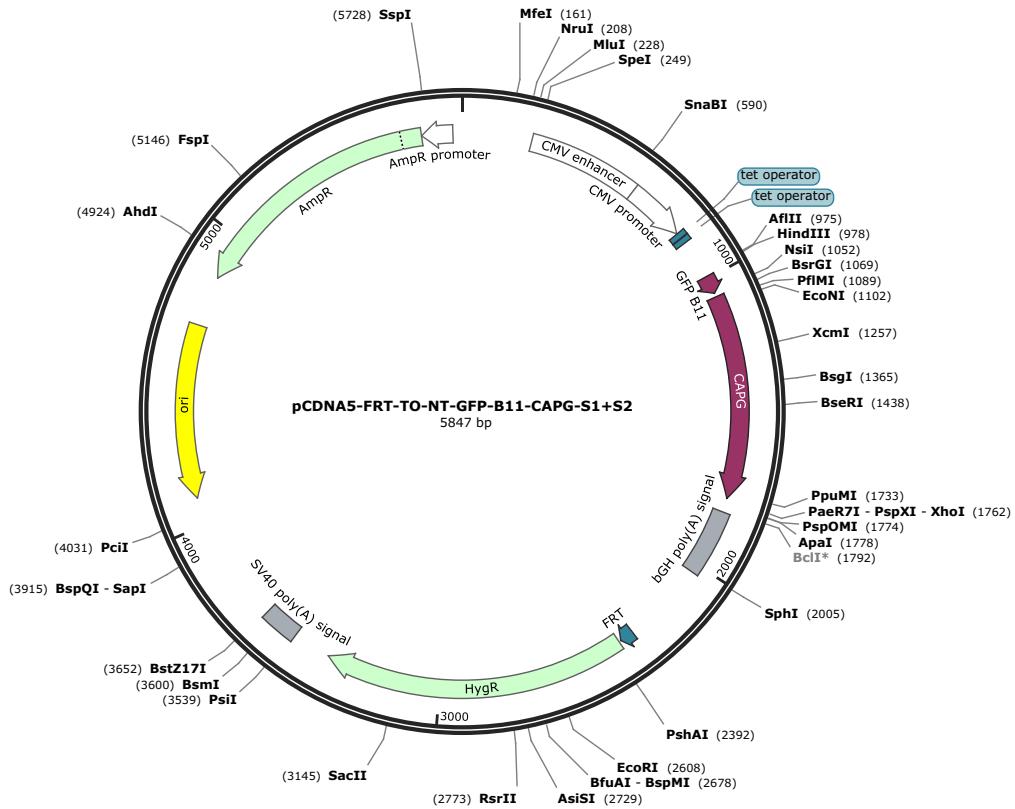
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>pCAPG-S3-CT-GFP-B11 (4375 bp)

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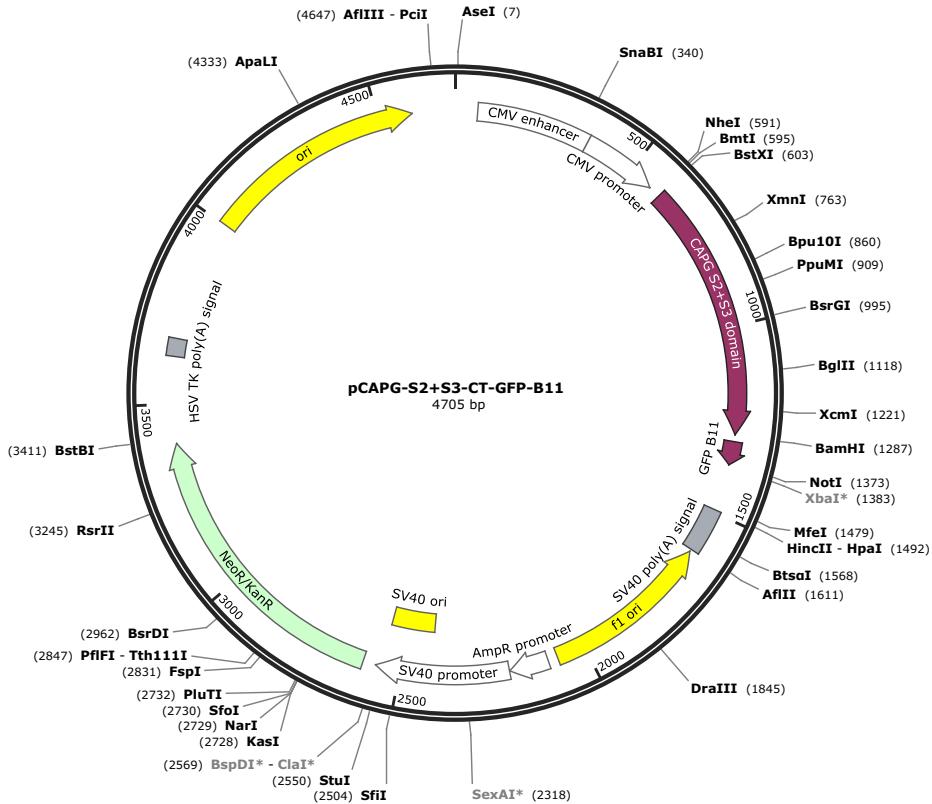
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>pCAPG-S2+S3-CT-GFP-B11 (4705 bp)

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