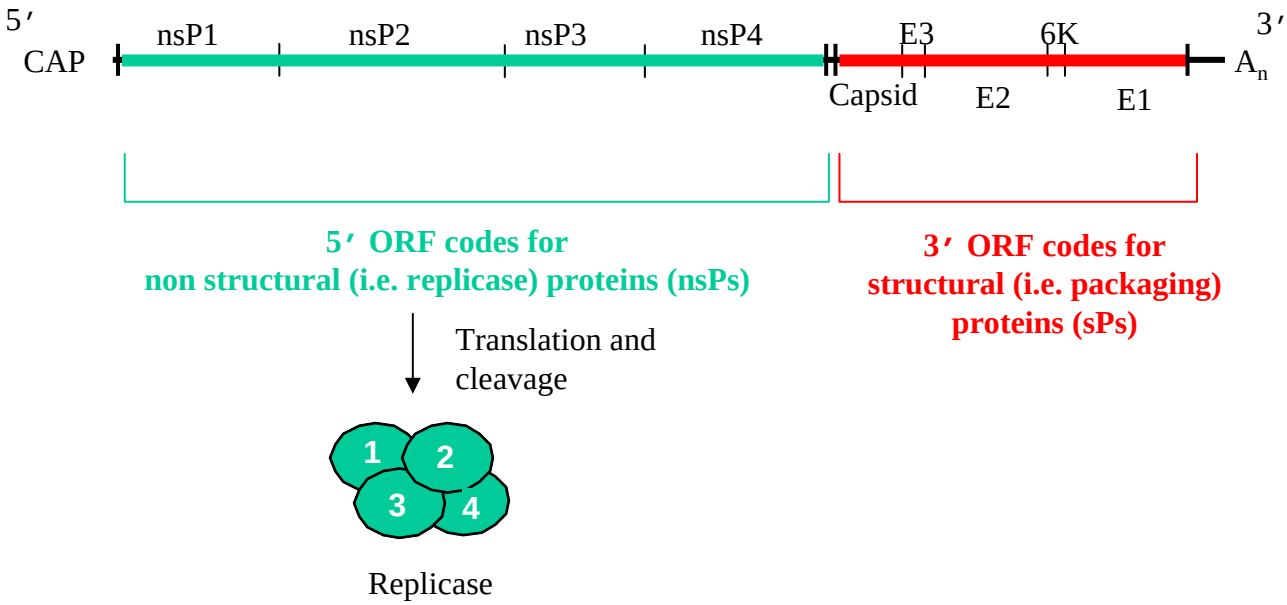
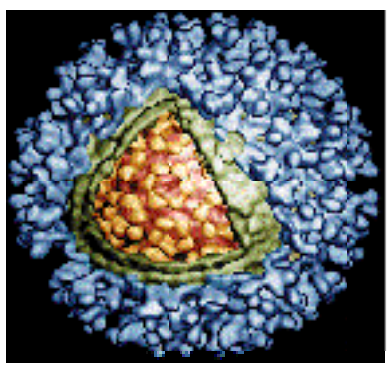
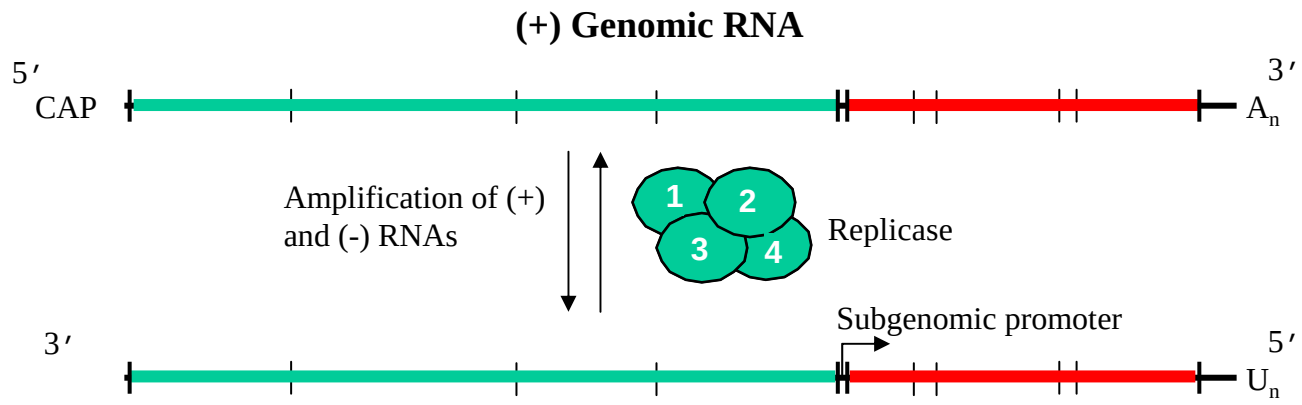
The (+) genomic RNA contains two open reading frames (ORFs).
Upon infection, the RNA is released, the 5' ORF is translated, and the protein product self-cleaved into four subunits, non-structural proteins 1-4 (nsP1-4). These combine to form the replicase. Intramolecular cleavage is performed by nsP2.



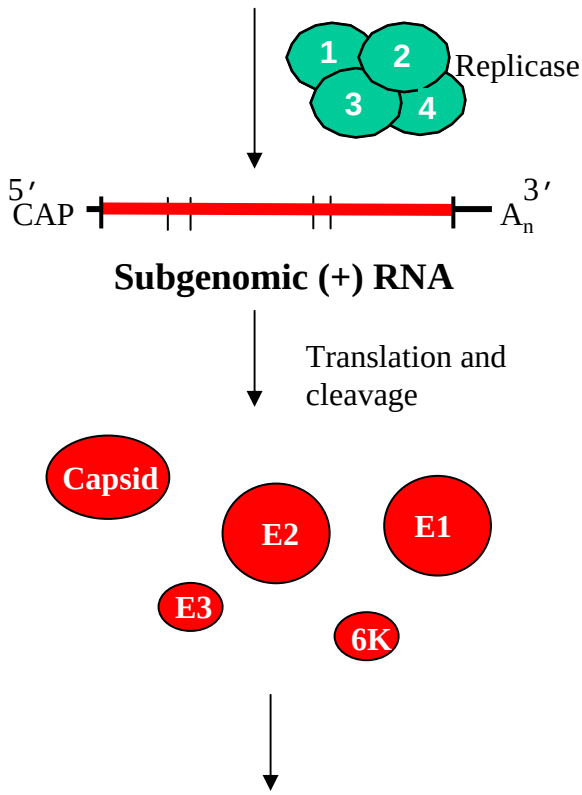


SFV virus
crystal structur e

Next, the replicase binds the 3'end of the (+) strand RNA and synthesizes a complementary (-) strand full length RNA.
The reverse reaction [(-) strand as template (+) strand as product] is also catalyzed by the replicase, resulting in massive amplification of the full length (+) and (-) strand RNAs.



Once the (-) strand is available, the replicase binds to a "subgenomic promoter" synthesizes a "subgenomic" 26S (+) RNA that contains the 3' ORF.
This 3' ORF encodes a protein that is self cleaved into the structural proteins (sPs). The order of these proteins on the 3' ORF are: Capsid, E3, E2, 6K, E1.

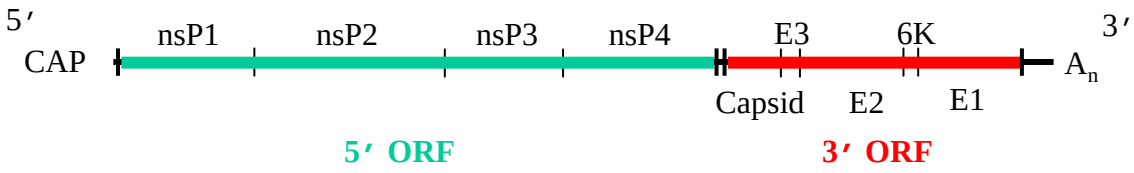


<u>Capsid</u>: forms the nucleocapsid around the (+) RNA genome. Is also a protease that cleaves the capsid from the remainder of the original polypeptide.
<u>E1, E2, E3</u>: Once capsid is removed, rest of polypeptide is transported to ER. Signal peptidase cleaves it into p62 (E3-E2 fusion), 6K, and E1. p62 and E1 heterodimerize (via E3 domain). At post-Golgi stage of transport, p62 is cleaved to E3 + E2. Three E1-E2 dimers form the spikes on the virus surface. E3 remains associated.
<u>6K</u>: Present in submolar quantities. Important for virus release.

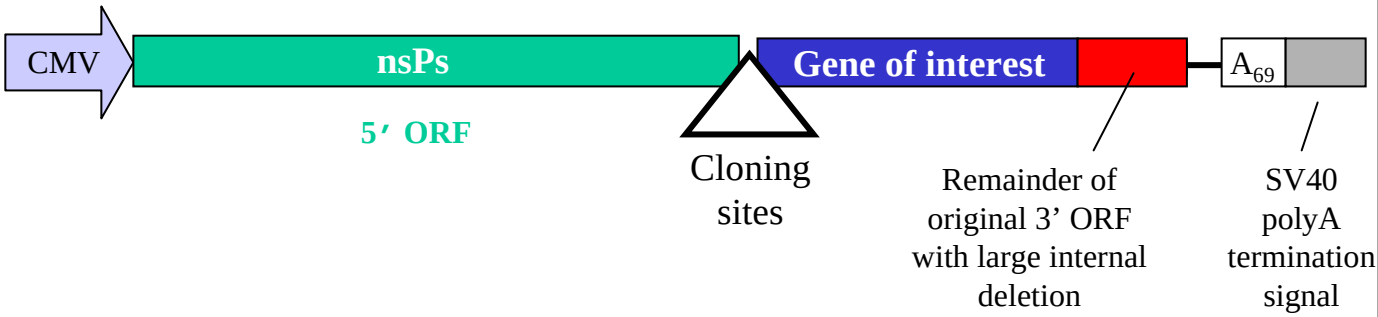
The capsid binds the (+) RNA packaging signal to form the nucleocapsid. Nucleocapsids diffuse to the cell surface. Glycoproteins reach cell surface through Golgi. Nucleocapsid and glycoproteins fuse and bud to complete virus life cycle.

Packaging of genomic (+) RNA
Budding of virus

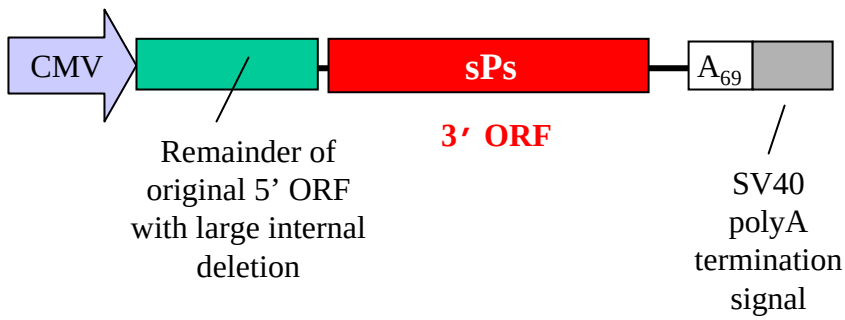
Wild type SFV genome (RNA)



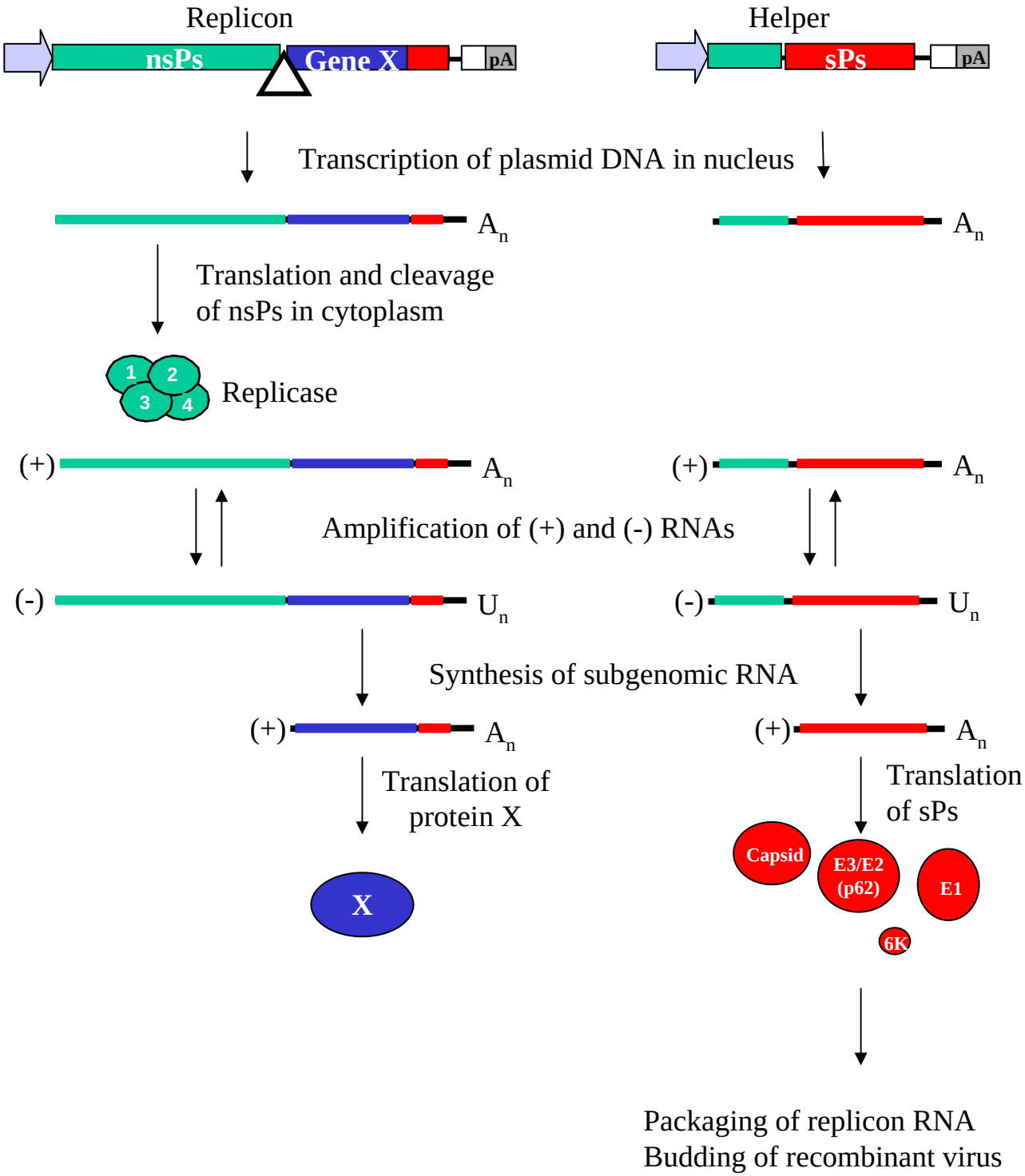
Replicon plasmid (DNA)



Helper plasmid (DNA)



To make virus, the replicon and helper plasmids are cotransfected (1:1 molar ration) into a cell line (originally BHK cells were used, which have to be electroporated, but now we transfect 293 cells by the simple calcium phosphate method, which works well).

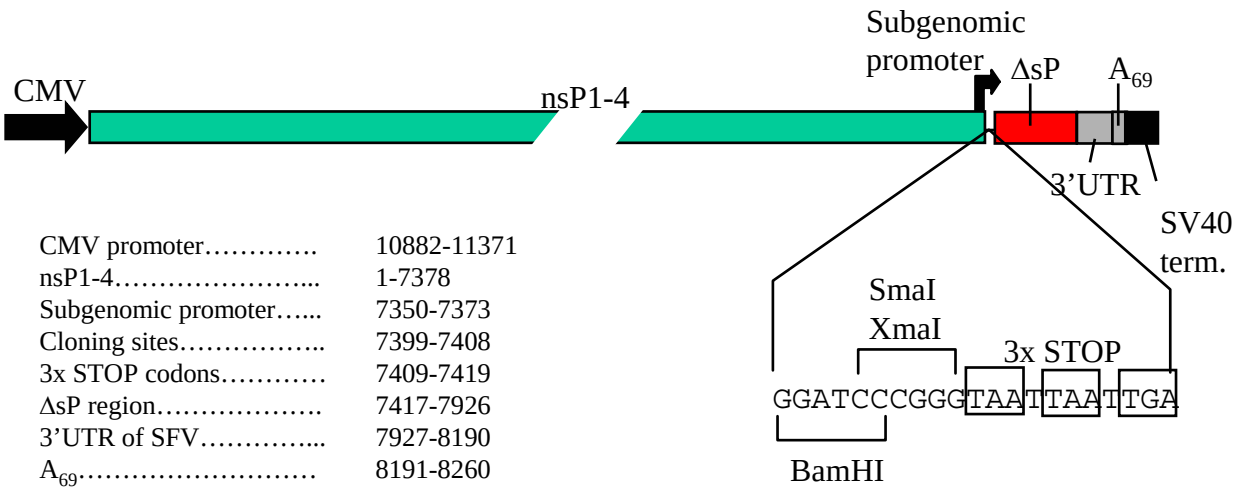


p62 (E3/E2) remains uncleaved due to three amino acid changes. To activate virus, p62 must be cleaved by chymotrypsin treatment. Upon infection, replicon RNA will be amplified and translated as depicted above, producing enough protein for you and your family to live on for years.

pSCA1
(11489bp)

This is the original vector we published in 1998 (LINK to pubmed).
The cloning site sucks, but you can easily throw in an oligo to add sites.

Full sequence of pSCA1 is here (**LINK to ascII - PDF**).



CMV promoter…….	10882-11371
nsP1-4……...	1-7378
Subgenomic promoter…..	7350-7373
Cloning sites……..	7399-7408
3x STOP codons……	7409-7419
ΔsP region…….	7417-7926
3'UTR of SFV……...	7927-8190
A_{69}……	8191-8260
SV40 termination signal…	8265-8506

•Drawing is to scale, although full length nsP1-4 region is not shown. Unique cloning sites are indicated.
• ΔsP is the non-functional structural protein ORF containing a large deletion.

Total length of nsP region is 7381 nucleotides. ORF starts at 86, ends at UAA at 7379-7381. ORF codes for 2431aa long polypeptide. Individual nsPs are generated by nsP2-mediated processing.

nsP1: 86-1696 (737aa)
nsP2: 1697-4090 (798aa)
nsP3: 4091-5536 (482aa)
nsP4: 5537-7378 (614aa)

Positions of nsPs are identical in the pSHAME vectors.

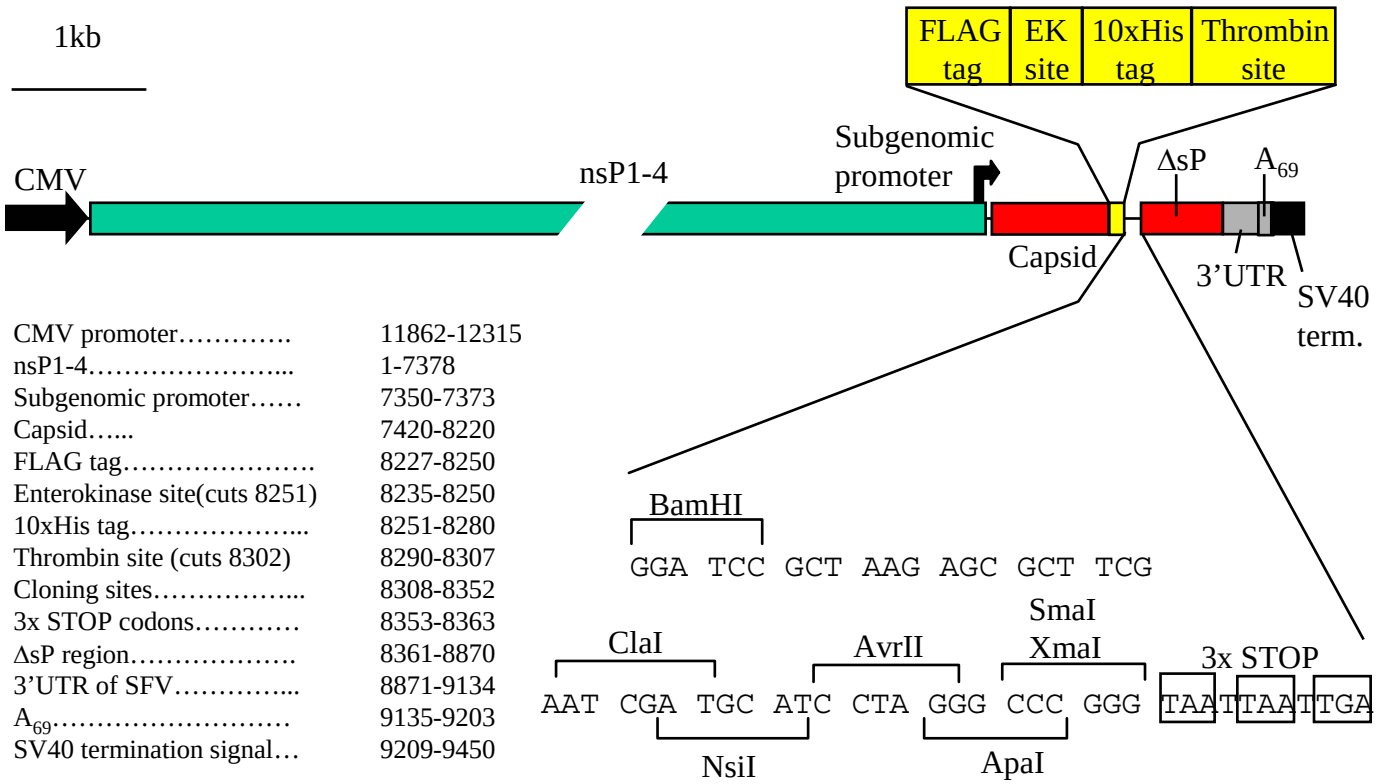
pSHAME1

(12433bp)

pSHAME2 (PLACE LINK HERE) is identical, but with 2 extra cloning sites between Bam and Cla (BlpI and BssHII)

Full sequence of pSHAME1 is here (LINK).

When cloning in the pSHAME series, YOU MUST INSERT YOUR cDNA SO THAT IT IS IN FRAME WITH THE UPSTREAM CAPSID-FLAG-10xHIS TAG. Ribosomes use the capsid AUG to start translating the subgenomic RNA, so if your cDNA is not placed in frame, your protein will not be made. The Capsid sequence contains a translation enhancer (up to 8-10 fold higher expression, 4-fold in our experience with pSHAME-lacZ). Immediately after translation, the Capsid self-cleaves, leaving behind your tagged protein.



CMV promoter…….	11862-12315
nsP1-4……...	1-7378
Subgenomic promoter……	7350-7373
Capsid…...	7420-8220
FLAG tag…….	8227-8250
Enterokinase site(cuts 8251)	8235-8250
10xHis tag……...	8251-8280
Thrombin site (cuts 8302)	8290-8307
Cloning sites……...	8308-8352
3x STOP codons……	8353-8363
ΔsP region…….	8361-8870
3'UTR of SFV……...	8871-9134
A_{69}……	9135-9203
SV40 termination signal…	9209-9450

•Drawing is to scale, although full length nsP1-4 region is not shown. Unique cloning sites are indicated.

•The FLAG tag can be cleaved with EK, and both FLAG &10xHis tags can be cleaved off using thrombin.

•The pSHAME cloning sequence is shown in triplets, corresponding to the reading frame

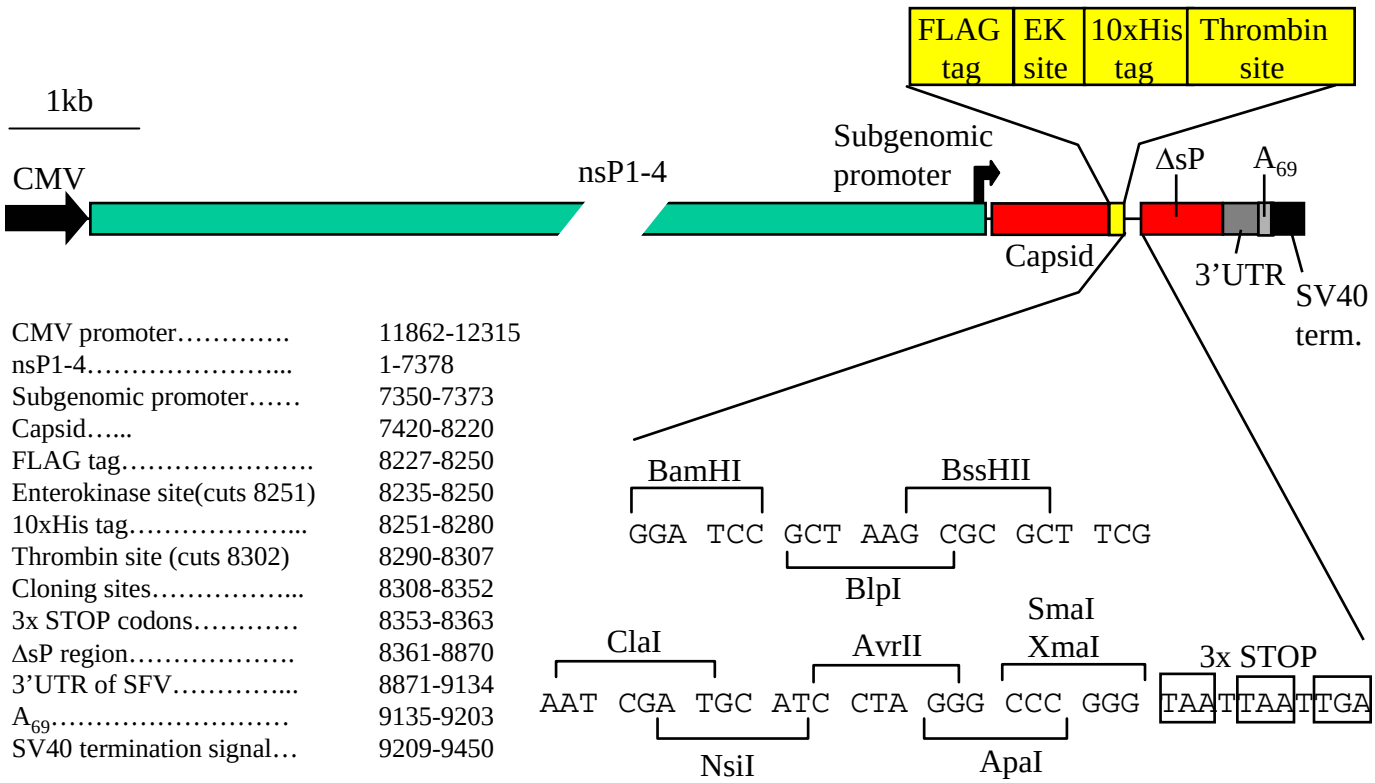
• ΔsP is the non-functional structural protein ORF containing a large deletion. Nucleotides 7421-7783 of the SFV genome are repeated twice in pSHAME, once in the Capsid segment (pSHAME 7421-7783) and again at the start of the ΔsP segment (pSHAME 8366-8728).

pSMART2a
(12433 bp)

pSMART1 is identical, but lacks cloning sites between Bam and Cla (BlpI and BssHII)

Full sequence of pSMART2a is here (LINK).

When cloning in the pSMART series, YOU MUST INSERT YOUR cDNA SO THAT IT IS IN FRAME WITH THE UPSTREAM CAPSID-FLAG-10xHIS TAG. Ribosomes use the capsid AUG to start translating the subgenomic RNA, so if your cDNA is not placed in frame, your protein will not be made. The Capsid sequence contains a translation enhancer (up to 8-10 fold higher expression, 4-fold in our experience with pSMART-lacZ). Immediately after translation, the Capsid self-cleaves, leaving behind your tagged protein.



CMV promoter…….	11862-12315
nsP1-4……...	1-7378
Subgenomic promoter……	7350-7373
Capsid…...	7420-8220
FLAG tag…….	8227-8250
Enterokinase site(cuts 8251)	8235-8250
10xHis tag……...	8251-8280
Thrombin site (cuts 8302)	8290-8307
Cloning sites……...	8308-8352
3x STOP codons……	8353-8363
ΔsP region…….	8361-8870
3'UTR of SFV……...	8871-9134
A_{69}……	9135-9203
SV40 termination signal…	9209-9450

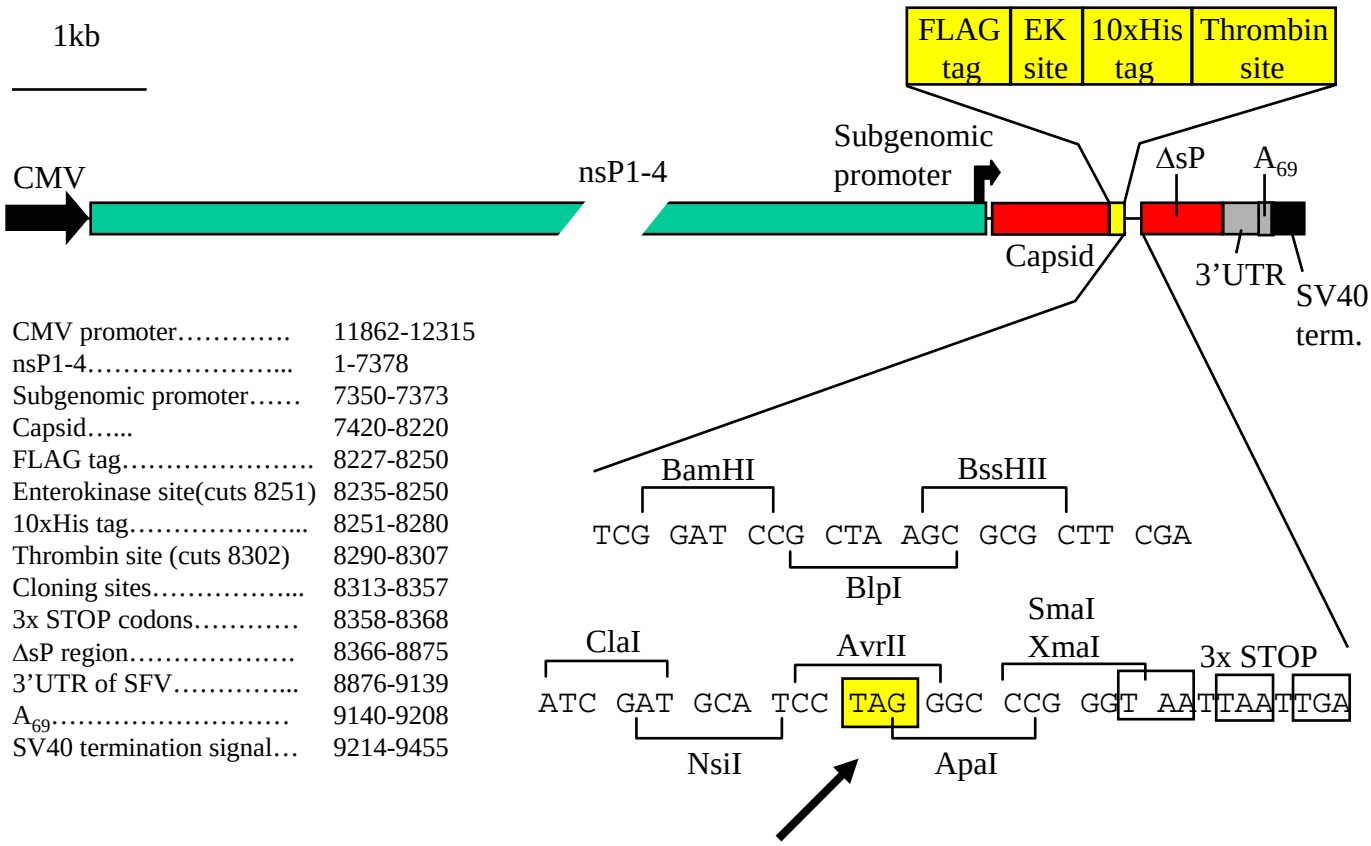
•Drawing is to scale, although full length nsP1-4 region is not shown. Unique cloning sites are indicated.
•The FLAG tag can be cleaved with EK, and both FLAG &10xHis tags can be cleaved off using thrombin.
•The pSMART cloning sequence is shown in triplets, corresponding to the reading frame
• ΔsP is the non-functional structural protein ORF containing a large deletion. Nucleotides 7421-7783 of the SFV genome are repeated twice in pSMART, once in the Capsid segment (pSMART 7421-7783) and again at the start of the ΔsP segment (pSMART 8361-8723).

pSMART2b
(12438 bp)

pSMART2a and 2b are the same, except for the reading frame of the cloning site.

Full sequence of pSMART2b is here (LINK).

When cloning in the pSMART series, YOU MUST INSERT YOUR cDNA SO THAT IT IS IN FRAME WITH THE UPSTREAM CAPSID-FLAG-10xHIS TAG. Ribosomes use the capsid AUG to start translating the subgenomic RNA, so if your cDNA is not placed in frame, your protein will not be made. The Capsid sequence contains a translation enhancer (up to 8-10 fold higher expression, 4-fold in our experience with pSMART-lacZ). Immediately after translation, the Capsid self-cleaves, leaving behind your tagged protein.

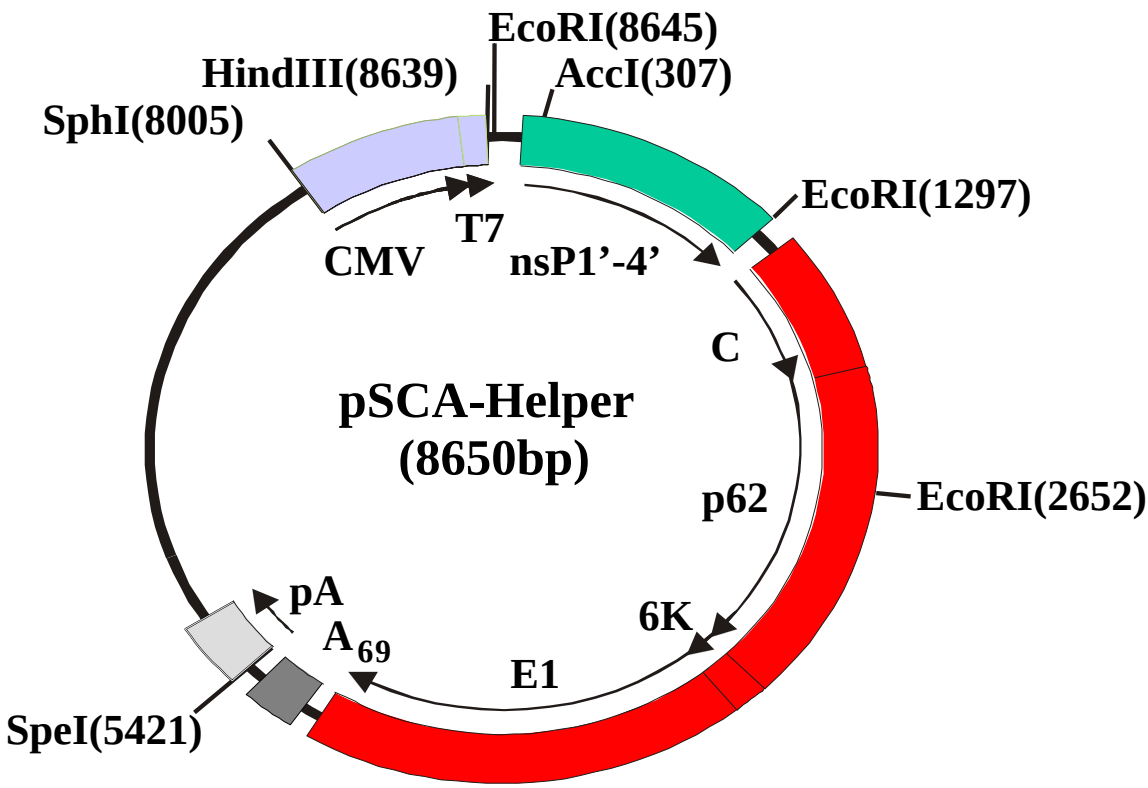


CMV promoter…….	11862-12315
nsP1-4……...	1-7378
Subgenomic promoter……	7350-7373
Capsid…...	7420-8220
FLAG tag…….	8227-8250
Enterokinase site(cuts 8251)	8235-8250
10xHis tag……...	8251-8280
Thrombin site (cuts 8302)	8290-8307
Cloning sites……...	8313-8357
3x STOP codons……	8358-8368
ΔsP region…….	8366-8875
3'UTR of SFV……...	8876-9139
A_{69}……	9140-9208
SV40 termination signal…	9214-9455

****Changed frame introduces earlier stop****

•Drawing is to scale, although full length nsP1-4 region is not shown. Unique cloning sites are indicated.
•The FLAG tag can be cleaved with EK, and both FLAG &10xHis tags can be cleaved off using thrombin.
•The pSMART cloning sequence is shown in triplets, corresponding to the reading frame
• ΔsP is the non-functional structural protein ORF containing a large deletion. Nucleotides 7421-7783 of the SFV genome are repeated twice in pSMART, once in the Capsid segment (pMART 7421-7783) and again at the start of the ΔsP segment (pSMART 8361-8723).

pSCAhelper



For complete sequence of pSCAhelper go here (**LINK**)

Position of sPs:

Capsid: 1329-2129 (267aa)
E3 (1st part of p62): 2130-2327
E2 (2nd part of p62): 2328-3593 (422aa)
6K: 3594-3773 (60aa)
E1: 3774-5087 (438aa)

; ### from DNA Strider Wednesday, August 19, 1998   6:35:27 PM
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; ### from DNA Strider Wednesday, May 24, 2000 9:15:47 AM
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