This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections).

Code(s) assigned:	***2008.083V***	(to be completed by ICTV officers)

Short title: New species in the genus Aphthovirus
(e.g. 6 new species in the genus *Zetavirus*; re-classification of the family *Zetaviridae* etc.)

Modules attached (please check all that apply):	**1** ☐	**2** ☐	**3** ☐	**4** ☐	**5** ☒
	6 ☐	**7** ☐			

Author(s) with e-mail address(es) of the proposer:

Nick Knowles (nick.knowles@bbsrc.ac.uk) on behalf of the Picornaviridae Study Group

ICTV-EC or Study Group comments and response of the proposer:

MODULE 5: **NEW SPECIES**

Code	***2008.083V***	(assigned by ICTV officers)

To create 1 new species assigned as follows:

Genus:	***Aphthovirus***
Subfamily:	
Family:	***Picornaviridae***
Order:	***Picornavirales***

Fill in all that apply. Ideally, species should be placed within a genus, but it is acceptable to propose a species that is within a Subfamily or Family but not assigned to an existing genus (in which case put "unassigned" in the genus box)

Name(s) of proposed new species:

Bovine rhinitis B virus

Argument to justify the creation of the new species:

If the species are to be assigned to an existing genus, list the criteria for species demarcation and explain how the proposed members meet these criteria.

Members of an aphthovirus species:
- share greater than 50% aa identity in the P1,
- share greater than 70% aa identity in the 2C + 3CD,
- share a natural host range,
- have a genome base composition which varies by no more than 1%,
- share a common genome layout.

ACID LABILITY
Virions are acid labile; FMDV particles are unstable below pH 6.8; Equine rhinitis A virus (ERAV) particles are unstable below pH 5.5. BRBV is unstable at low pH.

NUCLEIC ACID
There is a poly(C) tract close to the 5'-terminus of the genome. In FMDV it is located about 360 nt from the end, and varies in length from 100 to more than 400 nt. Current data suggests that the poly(C) tract in ERAV is shorter (± 40 nt, possibly less) and closer to the 5'-end. For BRBV the location and length of a putative poly(C) tract has not yet been determined. In the RNA of both species there is a series of pseudoknots on the 3'-side of the poly(C); the total 5' NTR is thus extremely long (1.1-1.5 kb). ERAV and FMDV differ by approximately 54% in nt sequence across the entire genome. BBRV differs by 46% (to FMDV) and 51% (to ERAV). Like FMDV and ERAV, the 5' UTR of BRBV contains a type II internal ribosome entry site (IRES) (Fig, 2).

PROTEINS
The major CPs of FMDV have the shortest chain lengths of any picornavirus (208-220 aa); those of ERAV are only slightly longer (226-248 aa) while those of BRBV are intermediate in size (219-235 aa). At the tip of the VP1 G-H loop of FMDV is the conserved integrin recognition motif, RGD. ERAV and BRBV both lack this feature. Differences in the P1 capsid between BRBV and FMDV are ~54-58% and between BRBV and ERAV are ~61-62%. See Table 1 for other proteins.

GENOME ORGANIZATION AND REPLICATION (see Fig. 1).
Translation starts at two alternative in-frame initiation sites, resulting in two forms of the L protein (Lab and Lb). L is a papain-like cysteine proteinase which cleaves itself from the virus polyprotein. These features are conserved in FMDV, ERAV and BRBV. The 2A polypeptide is very short (chain

Argument to justify the creation of the new species:

length=18 in FMDV; 18 in ERAV and 19 in BRBV), and is involved in NPGP-dependent polypeptide chain interruption at its C-terminus. The genome of FMDV encodes 3 species of VPg while ERAV and BRBV encode only one. Phylogenetic relationships are shown in Fig. 3.

BIOLOGICAL PROPERTIES

FMDV infects mainly cloven-hooved animals, but has been isolated from at least 70 species of mammals. Clinical manifestations of FMDV infections include foot-and-mouth disease (vesicular lesions), sometimes with associated acute fatal myocarditis in young animals; of ERAV, upper respiratory tract infections of horses; BRBV was isolated from the lungs of a calf. FMDV, ERAV and BRBV may produce persistent upper respiratory tract infections. FMDV infects cells by binding to integral membrane proteins of the integrin family through its VP1 G-H loop; heparan sulfate proteoglycans may also serve as receptors. Cap-dependent translation of host mRNA is inhibited by L^{pro}, which cleaves the host eIF-4G (FMDV, ERAV & BRBV) (see Fig, 4).

References:

Betts, A.O., Edington, N., Jennings, A.R. and Reed, S.E. (1971). Studies on a rhinovirus (EC11) derived from a calf. II. Disease in calves. *J. Comp. Pathol.* 81: 41-48.

Hollister, J., Vagnozzi, A., Knowles, N.J. and Rieder, E. (2008). Molecular and phylogenetic analyses of bovine rhinovirus type 2 show it is closely related to foot-and-mouth disease virus. *Virology* 373: 411-425.

Reed, S.E., Tyrrell, D.A., Betts, A.O. and Watt, R.G. (1971). Studies on a rhinovirus (EC11) derived from a calf. I. Isolation in calf tracheal organ cultures and characterization of the virus. *J. Comp. Pathol.* 81: 33-40.

Pilipenko, E. V., Pestova, T. V., Kolupaeva, V. G., Khitrina, E. V., Poperechnaya, A. N., Agol, V. I., and Hellen, C. U. (2000). A cell cycle-dependent protein serves as a template-specific translation initiation factor. *Genes Dev.* 14: 2028-2045.

Annexes:

Include as much information as necessary to support the proposal. The use of Figures and Tables is strongly recommended.

All tables and figures from Hollister *et al*., 2008

Table 1. Amino acid sequence identity (%) between BRV-2 (BRBV) and two representative members of the aphthoviruses*

	Amino acid identity	
Genome region	**% Relative to A24**	**% Relative to ERAV**
L	36	26
VP4	53	40
VP2	44	34
VP3	49	39
VP1	21	25
2A	67	63
2B	27	34
2C	52	44
3A	11	10
3B	52	24
3C	50	37
3D	64	47

* Accession numbers are A24Cru (AY 593768), BRV2 (EU 236594), ERAV (NP 653075)

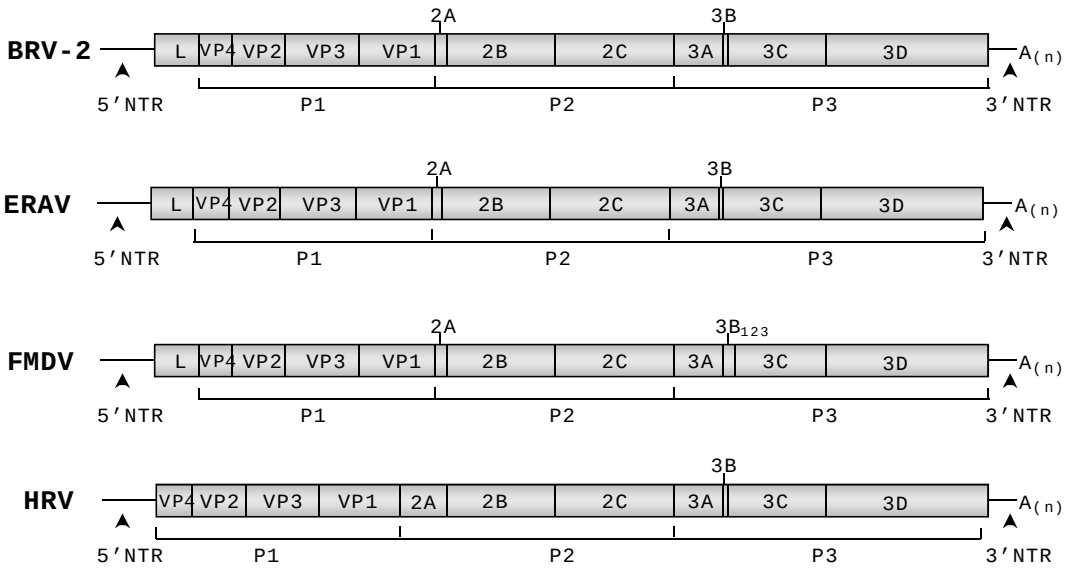


Fig. 1. Diagram of the genomic organization of BRV-2 (BRBV), FMDV ERAV and HRV genomes is shown with the individual protein coding regions boxed and shaded gray. Solid lines depict the 5' and 3' NTRs and are indicated by arrows. Gene regions VP4-VP1 (P1) encode capsid proteins while the remaining gene regions encode non-structural proteins (L, P2, P3).

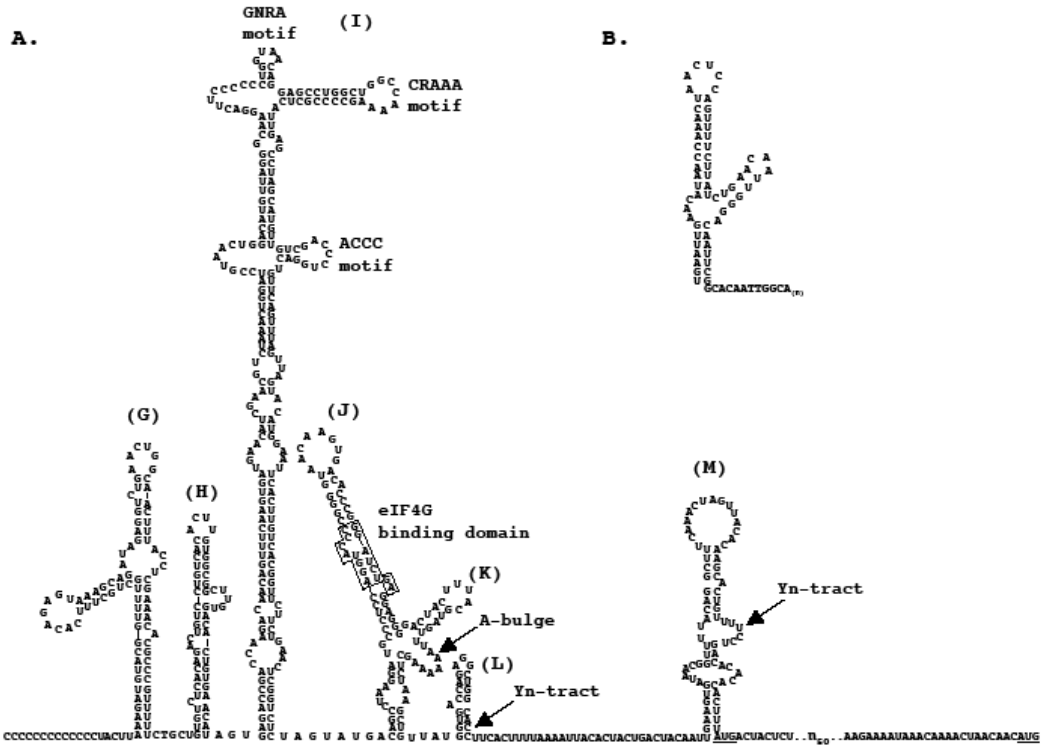
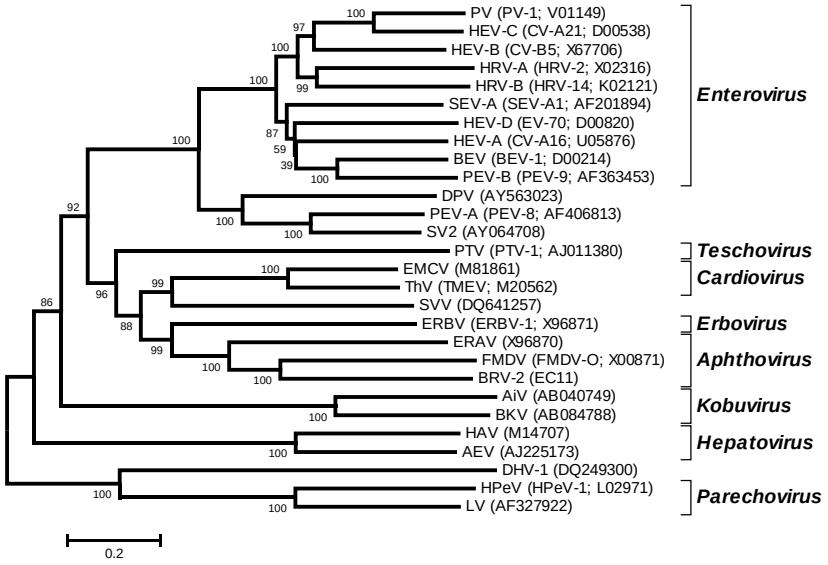
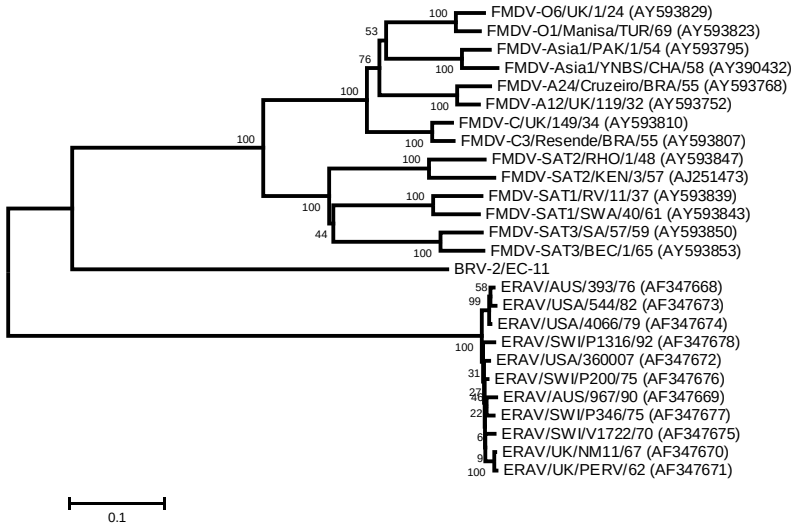


Fig. 2. Computer-predicted RNA secondary structure of the BRV-2 (BRBV) EC11 NTRs. A. Predicted RNA structure of the 5' NTR of BRV-2 (BRBV) EC11 following the poly(C) tract. Proposed domains are labeled G, H, I, J, K, L and M according to the nomenclature proposed by Pilipenko *et al.* (2000).The position of sequence motifs are indicated and denoted in bold. B. The predicted secondary structure in the 3' NTR of BRV-2 EC11.

A.


PV (PV-1; V01149)
HEV-C (CV-A21; D00538)
HEV-B (CV-B5; X67706)
HRV-A (HRV-2; X02316)
HRV-B (HRV-14; K02121)
SEV-A (SEV-A1; AF201894)
HEV-D (EV-70; D00820)
HEV-A (CV-A16; U05876)
BEV (BEV-1; D00214)
PEV-B (PEV-9; AF363453)
Enterovirus
DPV (AY563023)
PEV-A (PEV-8; AF406813)
SV2 (AY064708)
PTV (PTV-1; AJ011380)
Teschovirus
EMCV (M81861)
ThV (TMEV; M20562)
SVV (DQ641257)
Cardiovirus
ERBV (ERBV-1; X96871)
Erbovirus
ERAV (X96870)
FMDV (FMDV-O; X00871)
BRV-2 (EC11)
Aphthovirus
AiV (AB040749)
BKV (AB084788)
Kobuvirus
HAV (M14707)
AEV (AJ225173)
Hepatovirus
DHV-1 (DQ249300)
HPeV (HPeV-1; L02971)
LV (AF327922)
Parechovirus
0.2

B.


FMDV-O6/UK/1/24 (AY593829)
FMDV-O1/Manisa/TUR/69 (AY593823)
FMDV-Asia1/PAK/1/54 (AY593795)
FMDV-Asia1/YNBS/CHA/58 (AY390432)
FMDV-A24/Cruzeiro/BRA/55 (AY593768)
FMDV-A12/UK/119/32 (AY593752)
FMDV-C/UK/149/34 (AY593810)
FMDV-C3/Resende/BRA/55 (AY593807)
FMDV-SAT2/RHO/1/48 (AY593847)
FMDV-SAT2/KEN/3/57 (AJ251473)
FMDV-SAT1/RV/11/37 (AY593839)
FMDV-SAT1/SWA/40/61 (AY593843)
FMDV-SAT3/SA/57/59 (AY593850)
FMDV-SAT3/BEC/1/65 (AY593853)
BRV-2/EC-11
ERAV/AUS/393/76 (AF347668)
ERAV/USA/544/82 (AF347673)
ERAV/USA/4066/79 (AF347674)
ERAV/SWI/P1316/92 (AF347678)
ERAV/USA/360007 (AF347672)
ERAV/SWI/P200/75 (AF347676)
ERAV/AUS/967/90 (AF347669)
ERAV/SWI/P346/75 (AF347677)
ERAV/SWI/V1722/70 (AF347675)
ERAV/UK/NM11/67 (AF347670)
ERAV/UK/PERV/62 (AF347671)
0.1

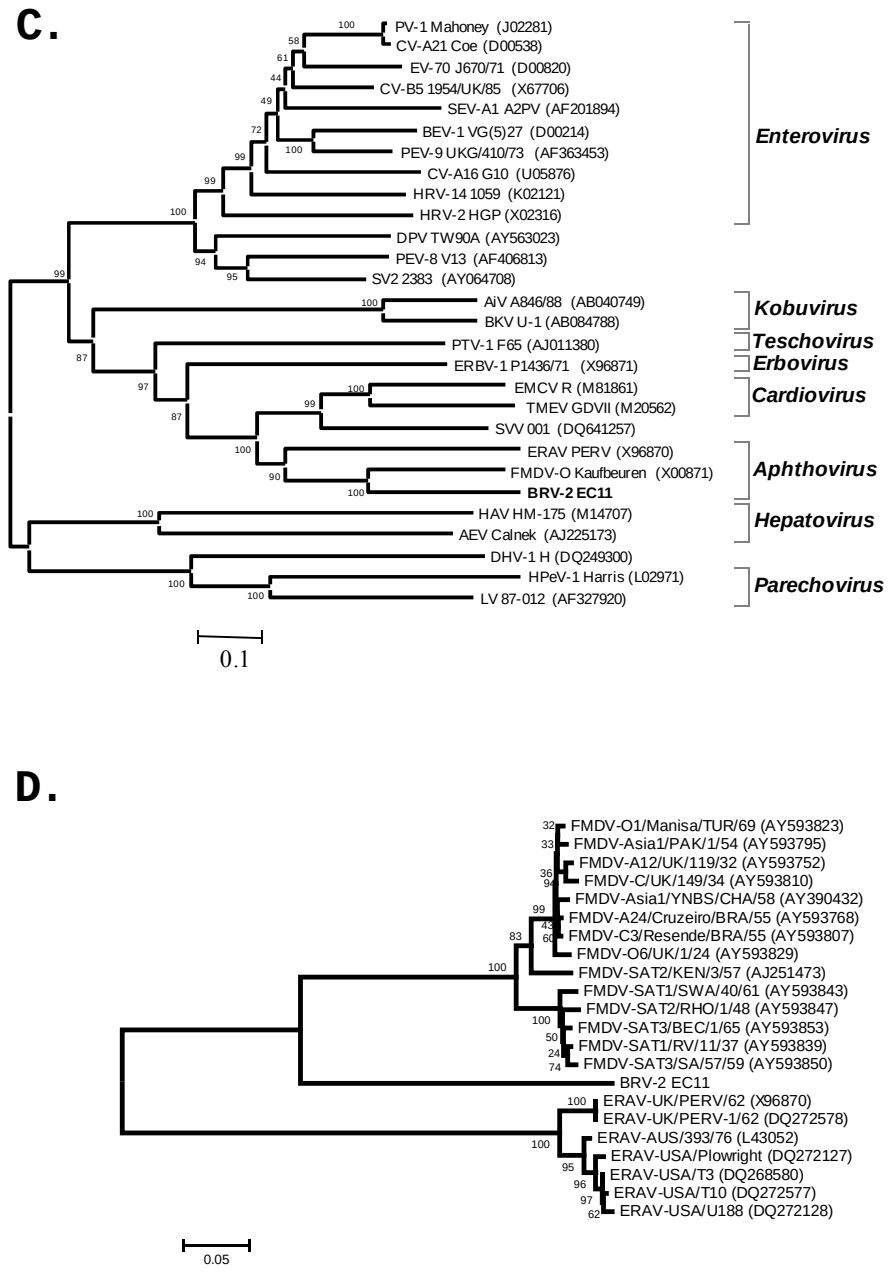


Fig. 3. Phylogenetic relationship of BRV-2 (BRBV) EC11 and other picornaviruses. A. Neighbor-joining trees of the P1 capsid protein of select picornaviruses from all genera, or B. from the *Aphthovirus* genus only. C. Neighbor-joining trees of the $3D^{pol}$ protein of picornaviruses representing all genera, or D. from the *Aphthovirus* genus only. Bootstrap analysis was applied using 1000 bootstrap replicates. The GenBank accession numbers and the percentages of bootstrap values are indicated. BRV-2 GenBank accession number, EU236594.

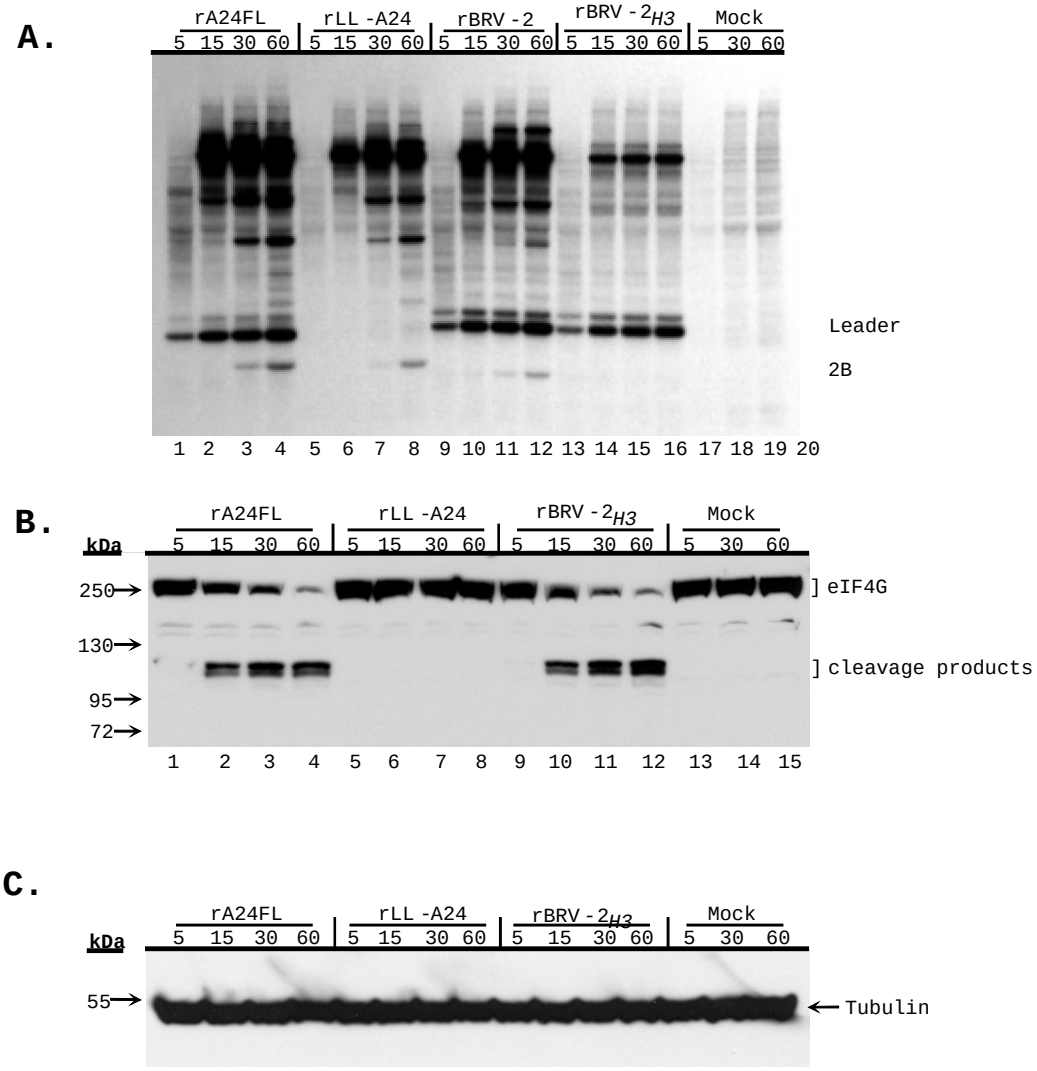


Fig. 4. Release of BRV-2 (BRBV) L protein from the viral polyprotein and cleavage of eIF4G. A. BKLF extracts were supplemented with 35S-methionine and programmed with RNA transcribed in vitro from the linearized plasmids pFMDV-A24 (lane 1-4), pLless-A24 (lane 5-8) and pBRV2 (lane 9-12 and 13-16). Samples taken at various times were separated by SDS-PAGE and autoradiography performed to visualize the labeled viral proteins. As a control extracts were incubated with mock RNA. Samples in the lanes marked rBRV2 H3(13-16) were programmed with RNA made from pBRV2 that was linearized with Hind III to preclude viral proteins downstream of 2B from being synthesized. The predicted position for L and 2B are indicated on the right. (B. and C.) Samples from the in vitro translation reactions described for Fig. 6A were separated by SDS-PAGE, transferred to PVDF membrane and analyzed by Western blotting with anti-eIF4G (B.) or α-tubulin (C.) as a control. The unprocessed eIF4G and its cleavage products are indicated on the right and the migration positions of molecular weight markers are indicated on the left.