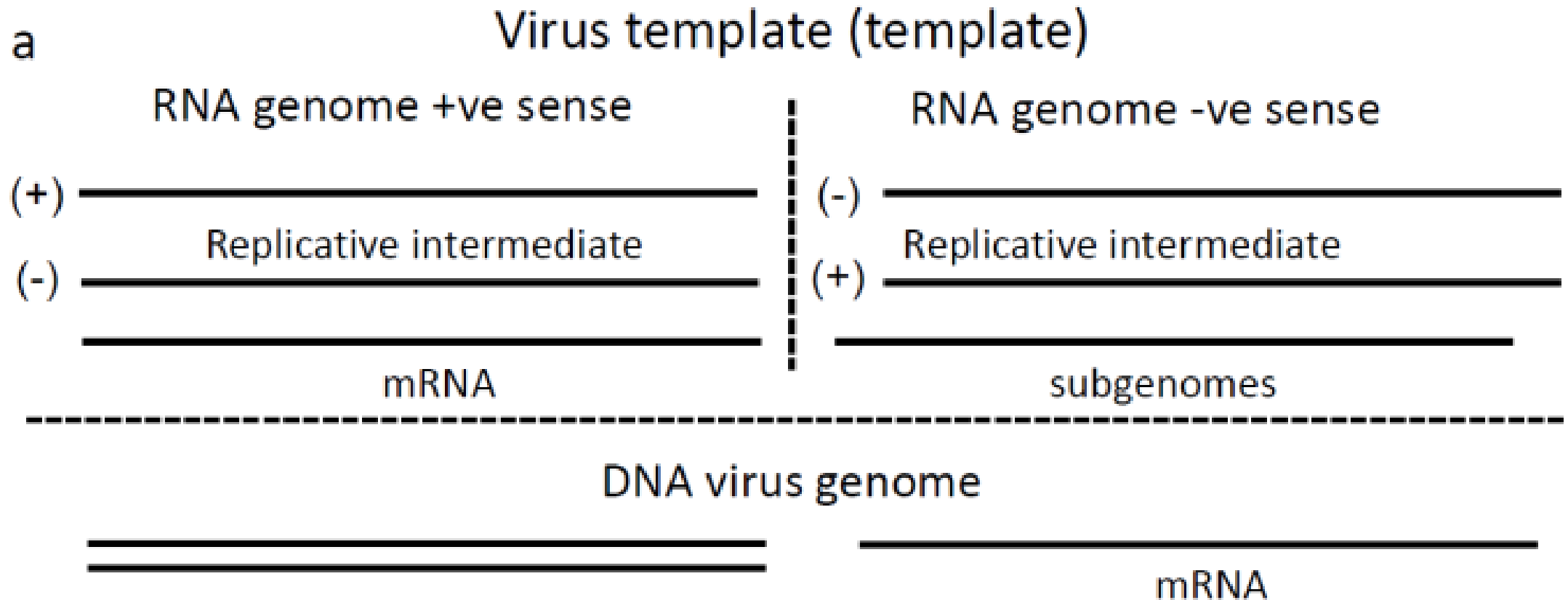


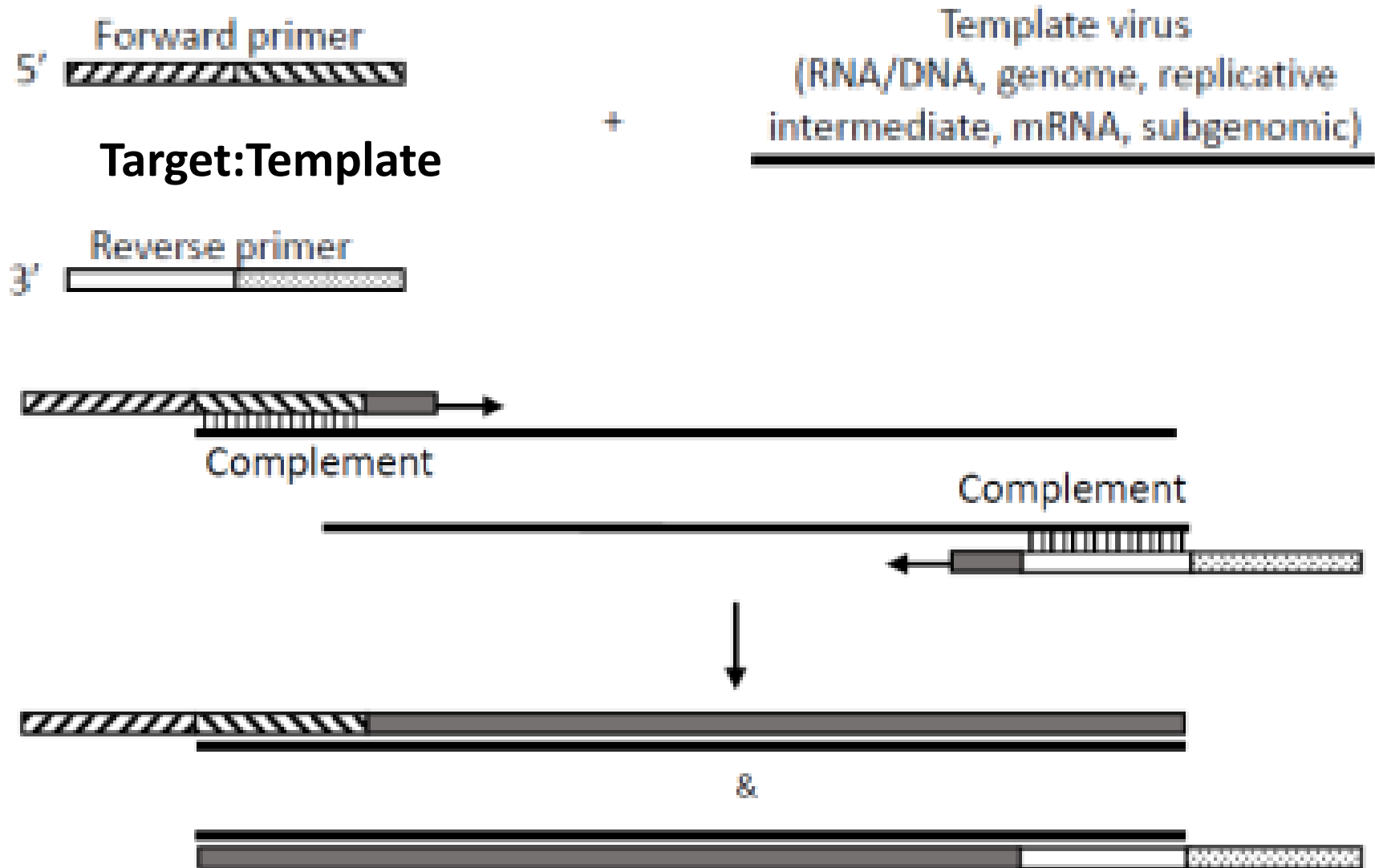
Virus mimicking artificial positive controls (ViMAPCs)

What do we need:

- A virus taxonomically related to the target (template virus)
- All components needed to run RT-PCR



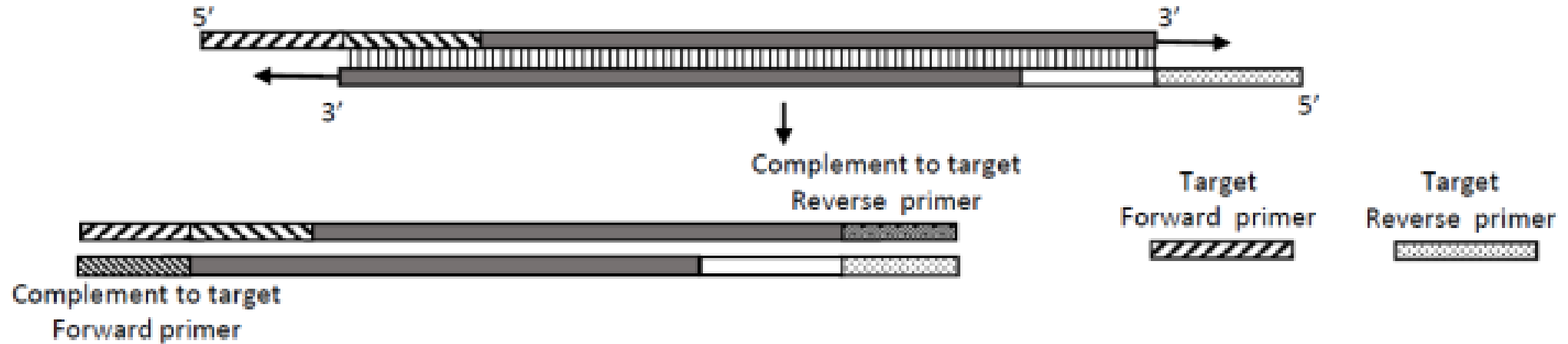
Step 1 – cDNA synthesis with chimeric primers and template virus



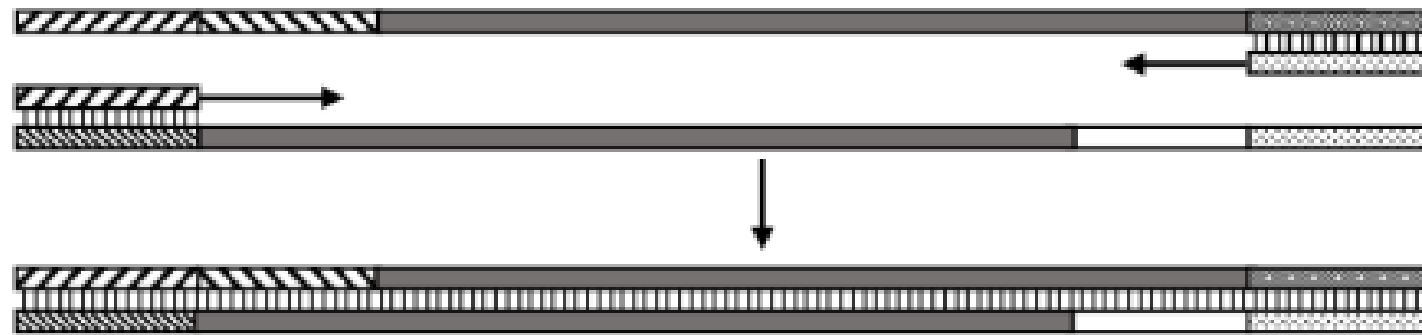
RNA/cDNA and/or DNA/cDNA hybrid with target tagged ends

Step 2 - PCR with target specific primers

First PCR cycle - DNA polymerase extends the cDNA and completes the ends



Subsequent PCR cycles – target primers amplify the ViMAPC template logarithmically



Amplicons with target ends and template backbones

d ViMAPC utilization as a positive control

True positive sample (3) is easily differentiated when compared to a ViMAPC (4)
ViMAPC (4) verifies successful target virus amplification

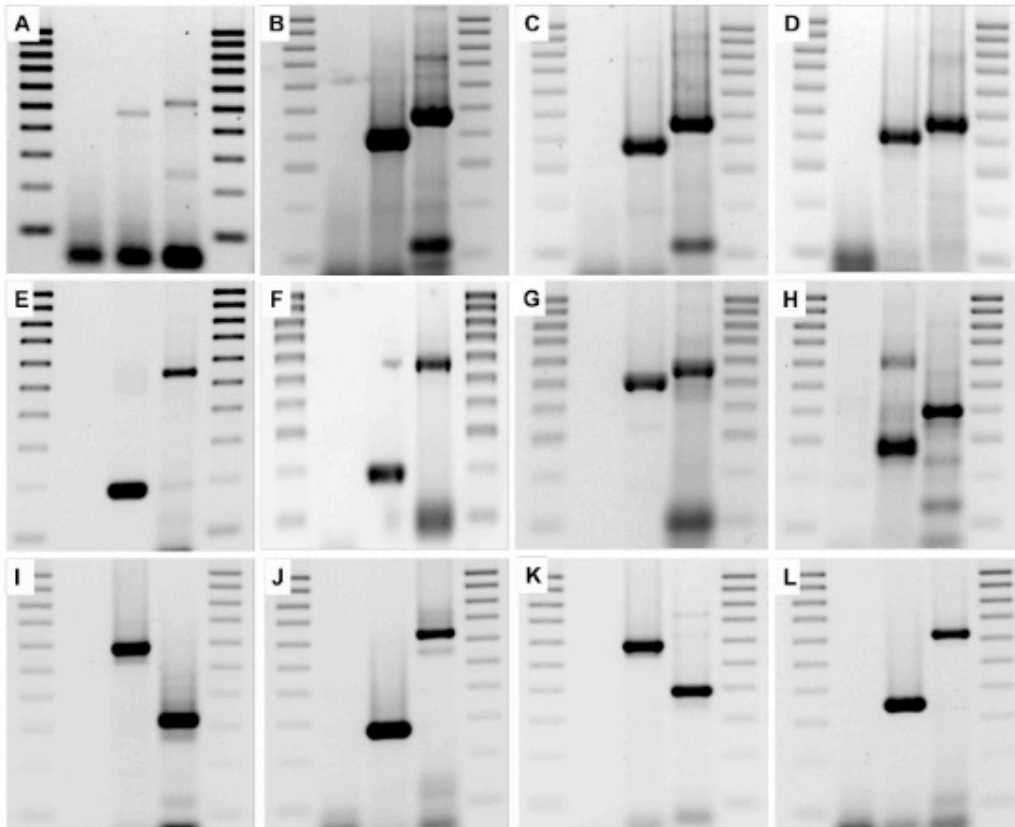


Detection PCR with target virus primers

- 1) Marker
- 2) Plant free from target virus
- 3) Plant positive for target virus
- 4) Virus-mimicking positive control (ViMAPC)

Lane 1 = 100 bp DNA marker; Lane 2 = Plant negative for target virus; Lane 3 = Plant positive for target virus; Lane 4 = ViMAPC and Lane 5 = 100 bp DNA marker.

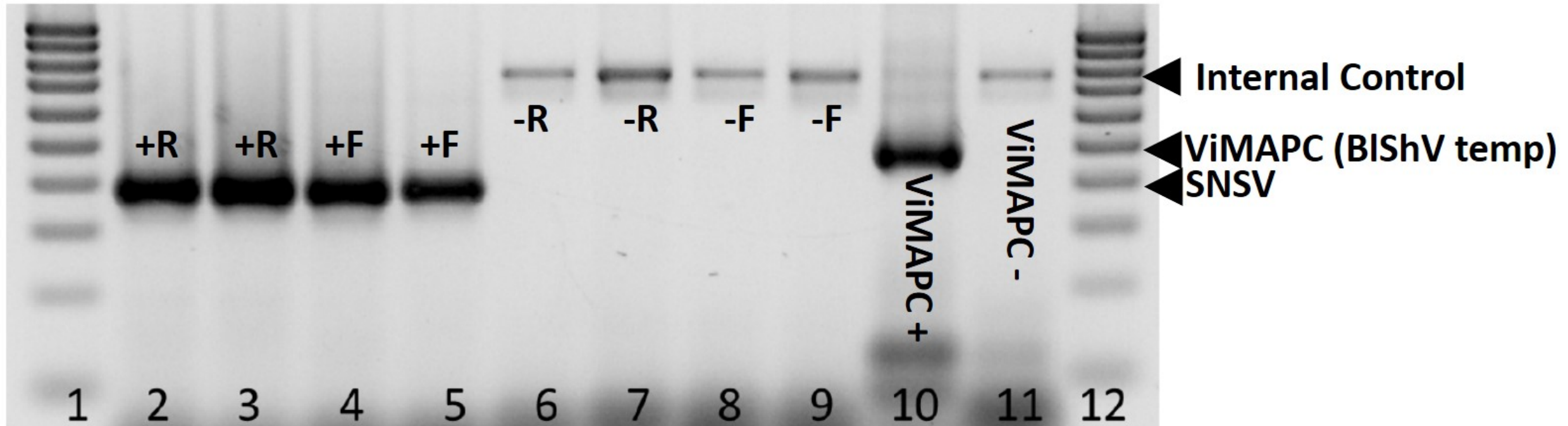
A. blackberry virus-F (BVF) using fig badnavirus-1 (FBV-1) as template; **B.** strawberry necrotic shock virus (SNSV) using blueberry shock virus (BIShV) as template; **C.** SNSV using blackberry chlorotic ringspot virus (BCRV) as template; **D.** BCRV using BIShV as template; **E.** peach rosette mosaic virus (PRMV) using tomato ringspot virus (ToRSV) as template; **F.** PRMV using tobacco ringspot virus (TRSV) as template; **G.** ToRSV using TRSV as template; **H.** rose rosette virus (RRV) using blackberry leaf mottle associated virus (BLMaV) as template; **I.** raspberry leaf blotch virus (RLBV) using RRV as template; **J.** RRV using RLBV as template; **K.** RLBV using BLMaV as template and **L.** BLMaV developed using RLBV as template.



Amplicon relative intensities: +/- 30%
of true positive as measured with ImageJ

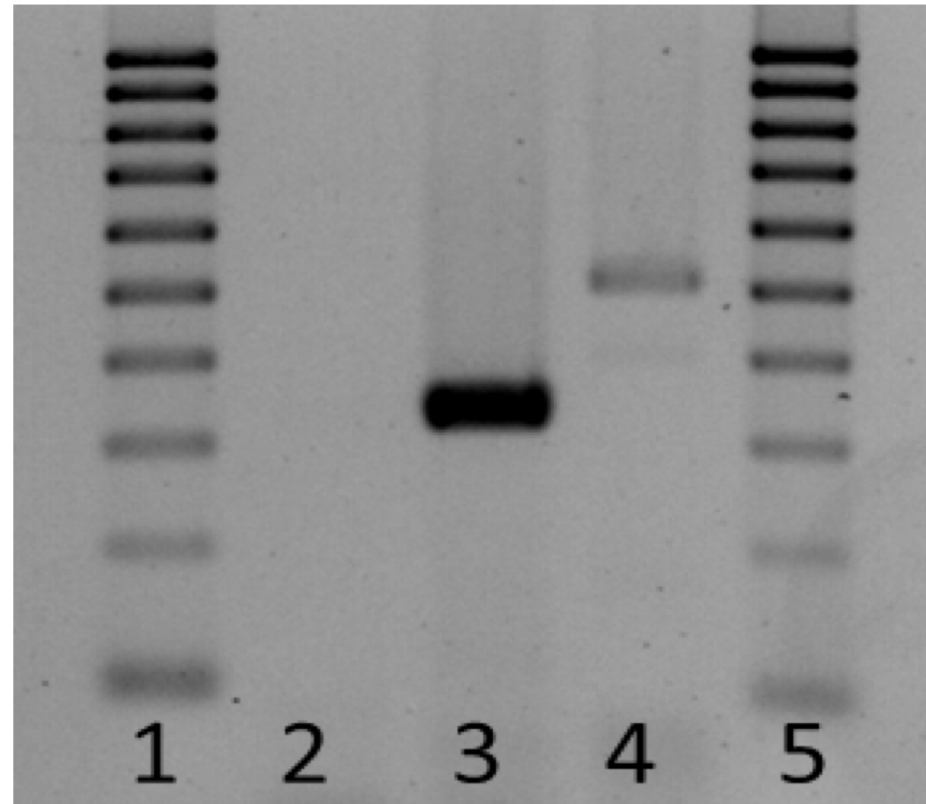
Application

Strawberry necrotic shock virus (SNSV) virus-mimicking artificial positive control (ViMAPC) developed using blueberry shock (BIShV)-infected material as template. Material infected with SNSV (*Rubus*: PI 691930 and PI 691943, *Fragaria* : PI 691784; PI 691800) and previously tested negative for the virus (*Rubus* : PI 691933 and PI 691936; *Fragaria* : PI 691758 and PI 691766) were tested alongside the ViMAPC (Lane 10) (supplementary table 3). All lanes show expected band sizes (supplementary table 1 and 2) (Thekke-Veetil et al., 2016). Lane 1 and Lane 12 represented 100 bp DNA marker.



Virus taxonomy is crucial !!!

Virus-mimicking artificial positive control virus mimicking artificial positive control ViMAPC for beet pseudo yellows virus (BPYV) family (genus *Crinivirus*,) developed using raspberry leaf mottle virus (RLMV, genus *Closterovirus*, family *Closteroviridae*) as template. Lanes 1 and 5: 100 bp DNA marker; lane 2: negative control; lane 3: positive control, 334 nucleotides; lane 4: BPYV ViMAPC, 513 nucleotides.



What are your questions?



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