

Evaluation of Pestivirus primers

1. The following primer and probe sets were evaluated:

Pan-Pestivirus primers

- *Pesti-11453-F*
- *Pesti-11607-R*

Atypical porcine pestivirus primer and probe set

- *APPV-NS5B-303F*
- *APPV-NS5B-385R*
- *APPV-NS5B-335-FAM* (probe)

2. Two databases were used to test the primers:

Includes 2261 sequences of **Pestiviruses NS5B** (BVDV 1 and 2, CSFV, Border disease among others; **2000 sequences**) and **Unclassified Pestiviruses**(BVDV 3, APPV, rat pestiviruses among others; **261 sequences**).

Only sequences equal or larger than 200 nucleotides were evaluated.

3. NCBI's e-PCR standalone application (<http://www.ncbi.nlm.nih.gov/tools/epcr/>) was used to evaluate the primers vs. the databases. Results are presented as a combination of hits with 0, 1, 2 and 3 mismatches.

4. Summary of results

Species coverage of individual pan-Pestivirus primers vs. Pestivirus database

The results for the most important pestiviruses are reported below:

Organism	Pesti-11453-F %Hits	Pesti-11607-R %Hits	Pesti-11453-F Hits	Pesti-11607-R Hits
Border disease virus	31.03	31.03	9 / 29	9 / 29
Bovine viral diarrhea virus 1	49.85	33.77	648 / 1300	439 / 1300
Bovine viral diarrhea virus 2	87.93	62.93	102 / 116	73 / 116
Bovine viral diarrhea virus 3	8.63	11.51	12 / 139	16 / 139
Classical swine fever virus	9.58	14.29	53 / 553	79 / 553
Porcine pestivirus 1	50	50	2 / 4	2 / 4

Organism	Pesti-11453-F %Hits	Pesti-11607-R %Hits	Pesti-11453-F Hits	Pesti-11607-R Hits
Porcine pestivirus isolate Bungowannah	25	25	1 / 4	1 / 4

Atypical porcine pestivirus primers

The previous results observed with PrimerBLAST were also observed with this process. There are only two out of four Atypical Porcine Pestivirus strains (Porcine Pestivirus 1) that are hit by both primers.

Notably, there are **9 strains of BVDV3** that are hit by the **APPV-NS5B-303F** forward primer (all of them with 3 mismatches). **The reverse primer, as well as the probe, only match the two APPV strains** that were identified as being the only products of this oligo set (NCBI accessions KU194229.1 and KR011347.1). None of the primers nor does the probe hit any of the Porcine pestivirus isolate Bungowannah sequences. Thus, we can say that the assay is specific to two strains of Porcine Pestivirus 1 and that other pestiviruses will likely not be detected.