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)

1. Two databases were used to test the primers:

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

Only sequences equal or larger than 200 nucleotides were evaluated.

1. [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.
2. 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 | 100 | 100 | 2 / 2 | 2 / 2 |

### Atypical pestivirus primers

The previous results observed with PrimerBLAST were also observed with this process. There are only two 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). Thus, we can say that the assay is specific to two strains of APPV and that there are no concerns of amplifying other pestiviruses.