Hi @Vida @Manu V.,

We tried Primalscheme today to design a primer pool for Tetraparvovirus genome. We used 6 sequences from CHRF and 22 from NCBI (selected considering >=95% coverage & >=90% identity with Ref NC\_007018.1). As per the instruction, we aligned these sequences using ClustalO and uploaded them on the primalscheme. Amplicon size was set to 400 bp.

We considered the alignment of these 28 sequences as v1. The results showed 16 sets of primers with 91% genome coverage. However, primalscheme excluded 16 sequences due to failed alignment (incl. 6 CHRF sequences, because of shorter length, 4860-4911 bp).

So, we discarded 12 short-length sequences (incl. 6 from CHRF) from v1. Rest 16 sequences were aligned again (named it v2). The primalscheme showed 17 primer sets this time, with 93.41% genome coverage.

As it was still <95% coverage, we discarded 7 sequences from v2 and kept only 5 complete or near-complete genomes (named it v3). After alignment and analysis, the webtool gave us 17 primer sets with 94.55% genome coverage.

The attached zipped file has all alignment files and results (individual folders for v1, v2, and v3). Please let us know your thoughts.