European Union Reference Laboratory for Fish and Crustacean Diseases

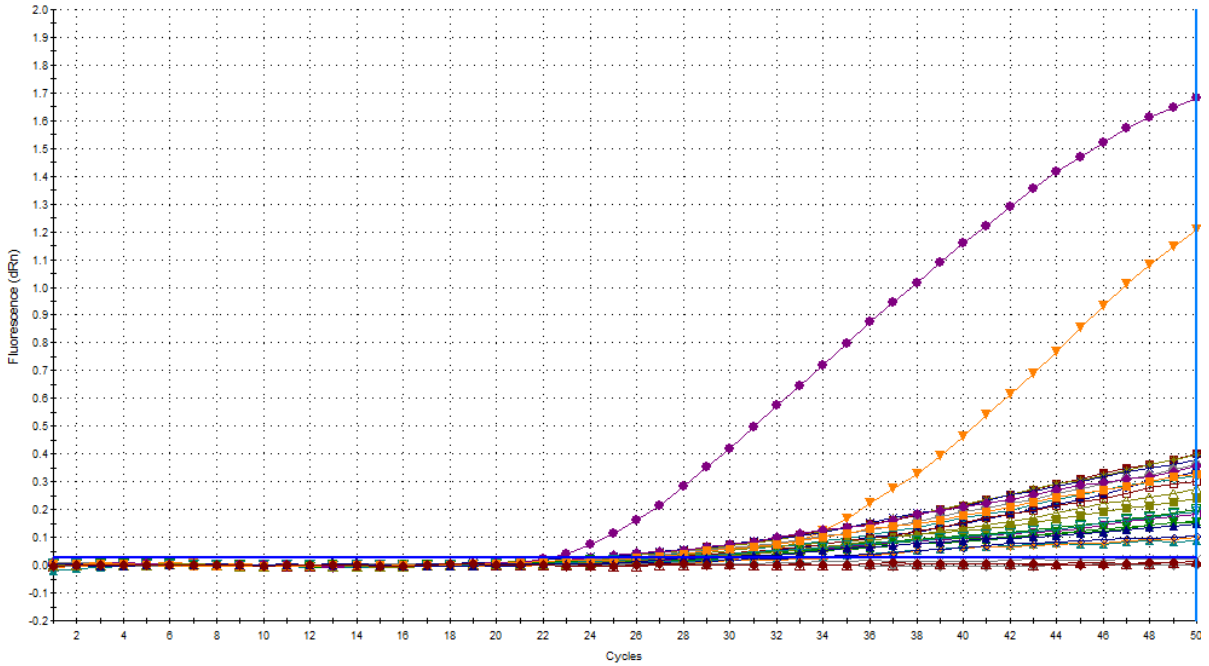
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Information regarding molecular detection of IHNV in Denmark

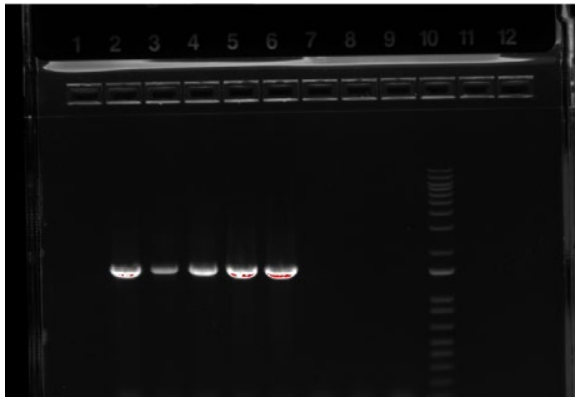
RT-qPCR performances and variation in targeting region of the N-gene

IHNV diagnostics in Danish cases

Recommended RT-qPCR with atypical shape, linear amplification



Samples are clearly positive using end-point RT-PCR targeting the N-gene (OIE) and the G-gene and sequenced



Test of RT-qPCR in agarose gels indicate that the lack of sensitivity is caused by possible mutation in the area of probe hybridization

What is happening to Purcell et al. 2013 RT-qPCR?

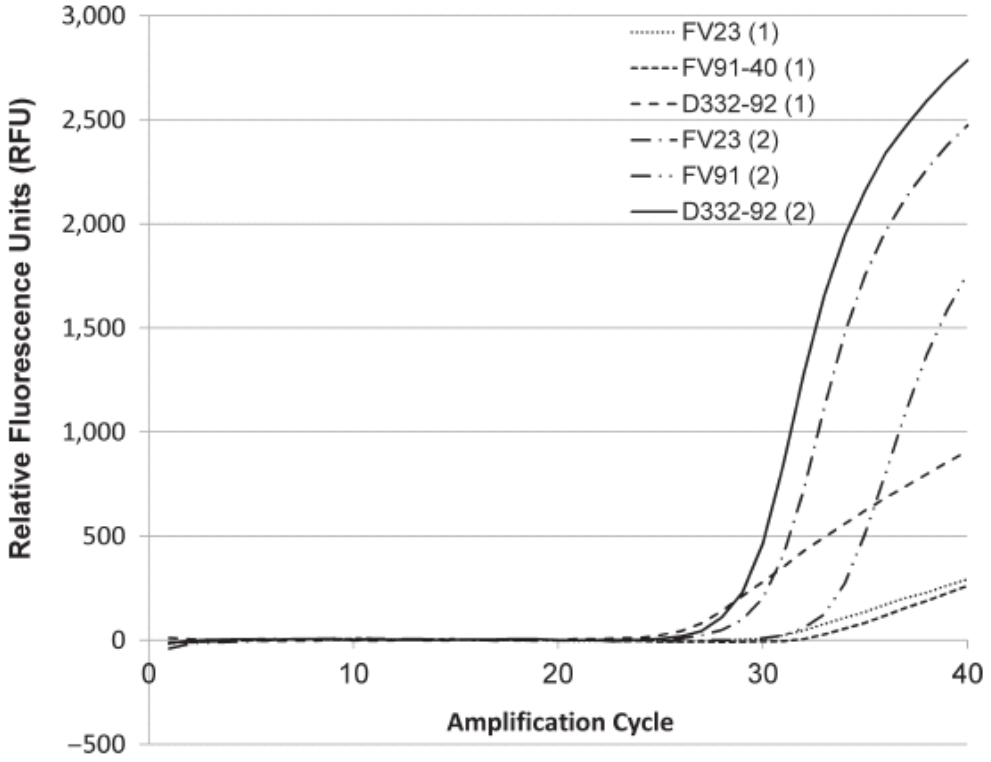


FIGURE 1 Amplification curves generated by the original IHNV RT-qPCR protocol provided by Purcell et al. (2013) using an MGB TaqMan® probe (RFU values <1,000) (1) in comparison with the improved protocol designed in this study using a TaqMan® probe run with a modified thermal profile in a one-step protocol (RFU values >1,000) (2)

Hoferer et al. 2019 report few German isolates where the Purcell et al. 2013 method shows a lower sensitivity

Improvement of a diagnostic procedure in surveillance of the listed fish diseases IHN and VHS

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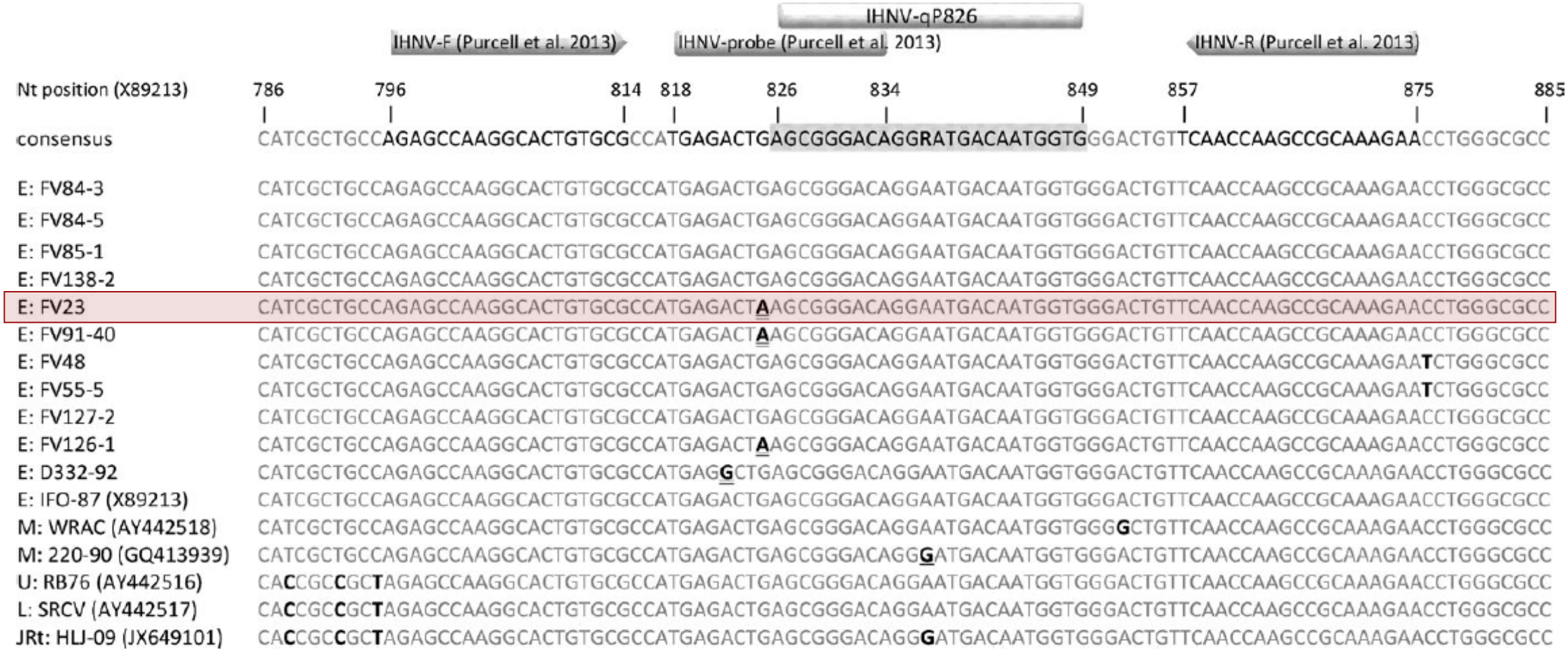
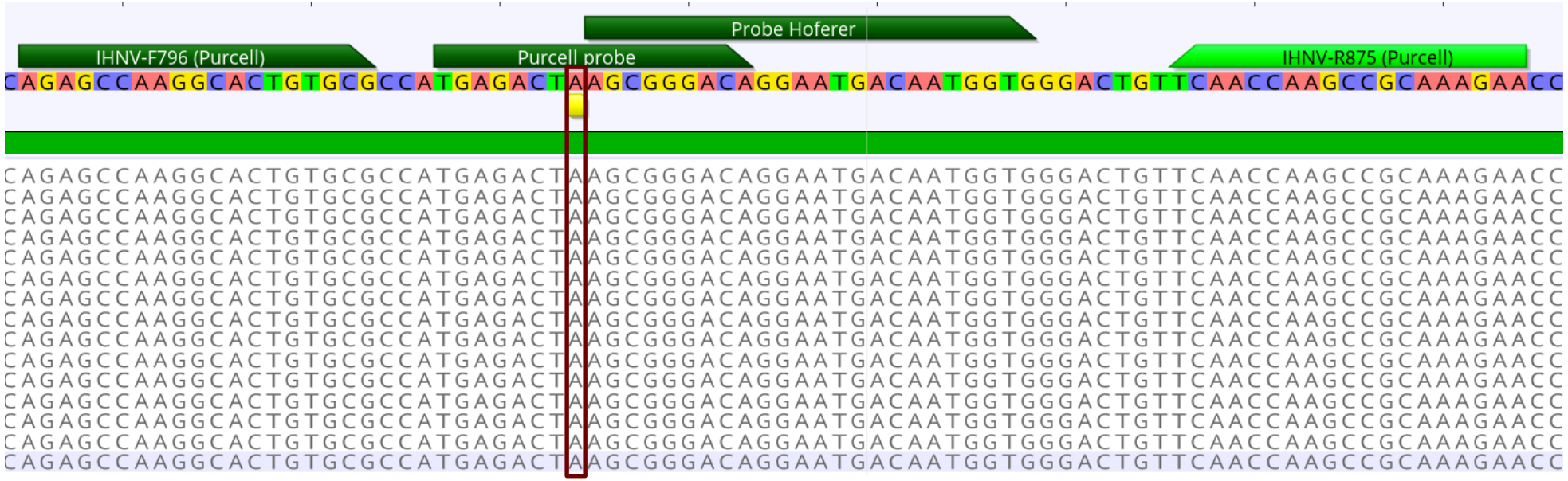
Modification of RT-qPCR method



Danish Isolates – N Gene sequences

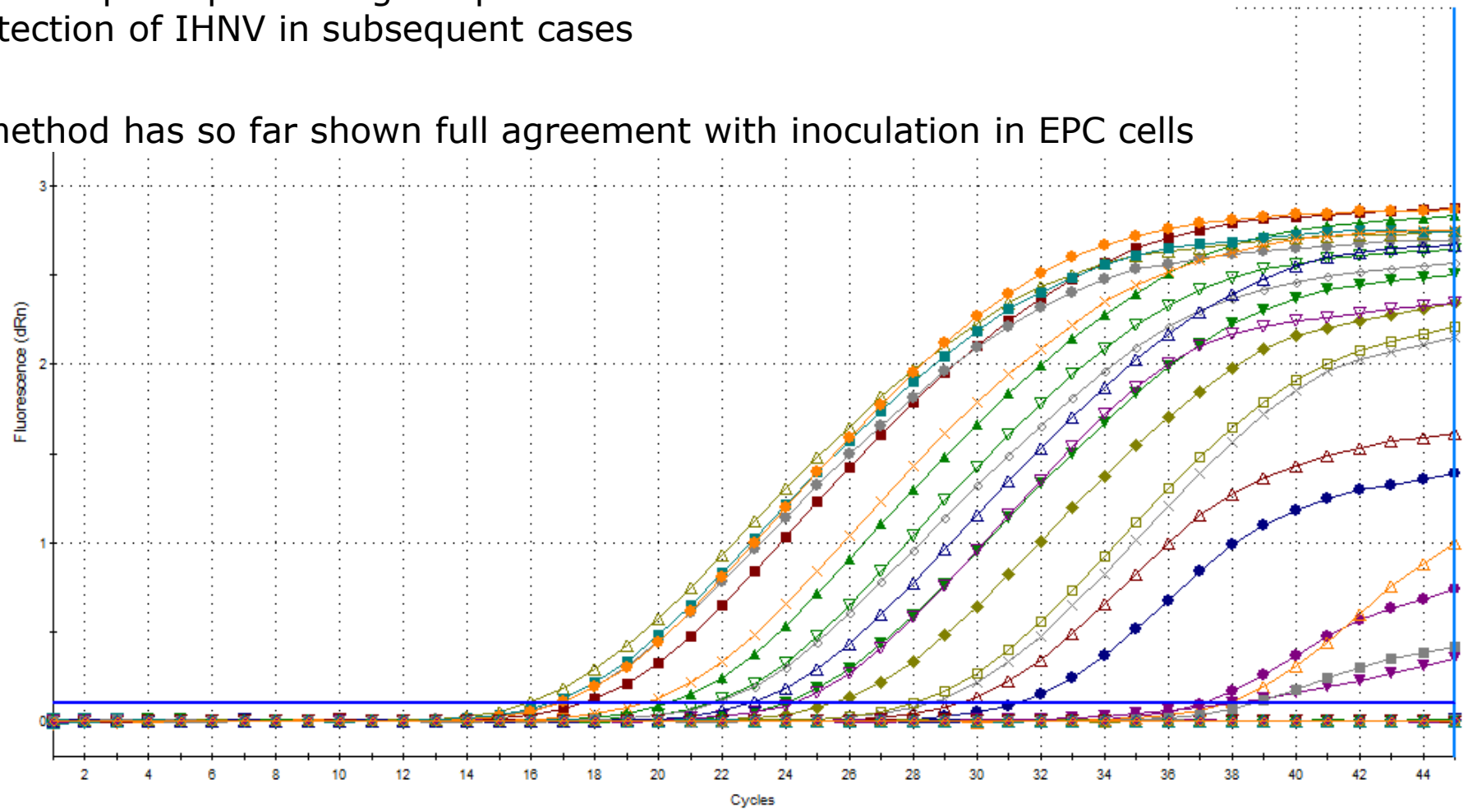
Danish IHNV isolates have a mismatch with Purcell probe (A instead of G), which dramatically reduces the sensitivity of detection



RT-qPCR results new probe (from Hoferer 2019)

The one-step RT-qPCR using the probe from Hoferer 2019 has been used for detection of IHNV in subsequent cases

This method has so far shown full agreement with inoculation in EPC cells



Current recommendation for detection of European IHNV viruses

- Primers:

  IHNV N 796F AGAGCCAAGGCACTGTGCG (Purcell et al. 2013)

  IHNV N 875R TTCTTTGCGGCTTGGTTGA (Purcell et al. 2013)

- Probe:

  IHNV-qP826 AGCGGGACAGGRATGACAATGGTG (Hoferer et al. 2019)