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Comparative analysis of sequences from PT 2013

Annual Meeting of the National Reference Laboratories

May 28-29th 2014

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AP+Sp-

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Genotyping and sequencing

- 43 laboratories participated in the proficiency test
- Sequencing mandatory for EHNV
- \bullet Sequencing not mandatory, but recommended for VHSV and IHNV
- Sequencing highly recommended for ISA (HPR0)
- Furthermore, several laboratories sequenced SVCV and IPNV

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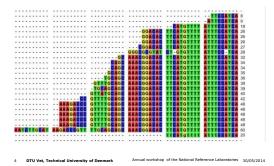
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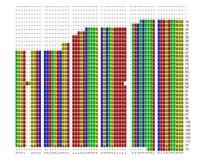
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EHNV

- 41 laboratories sent in 43 sequences.
- 1 sequence mapped to the Neurofilament gene, while the rest mapped to the MCP gene.
- 28 sequences contained no errors/wobble bases at all.
- All sequences were written in as EHNV, but one sequence had 99% similarity with European Sheatfish virus and 98% similarity with EHNV.
- One sequence only mapped with 88% similarity to EHNV.
- 6 sequences contained only 1 error, while 7 sequences contained more than one.
- Errors were indels or substitutions
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Common mistakes

- If primersites are not deleted
- If isolates are sequenced only in one direction. The EURL advises that isolates be sequenced in both the forward and reverse direction. If isolates are cloned it is recommended that at least 3 clones are sequenced.
- Improper reading/analysis of trace files

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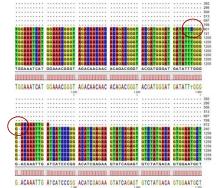
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VHSV

- 13 laboratories sequenced the G-gene
 - 8 sequenced the full G-gene, 1 did not assemble sequences
 - 5 labs sequenced between 500 and 800 nt
 - 3 sequences contained mistakes, always at the end

• 12 laboratories sequenced the N-gene

- Sequences varied between 264 and 723 nt
- 5 sequences contained mistakes, always at the ends

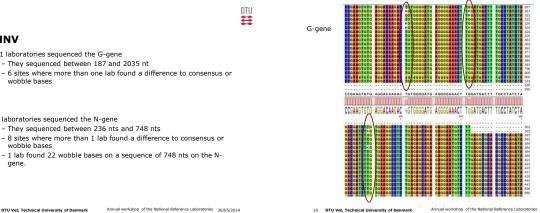


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- 1 lab found 22 wobble bases on a sequence of 748 nts on the N-

IHNV

• 21 laboratories sequenced the G-gene - They sequenced between 187 and 2035 nt

• 8 laboratories sequenced the N-gene

- They sequenced between 236 nts and 748 nts

wobble bases

gene.

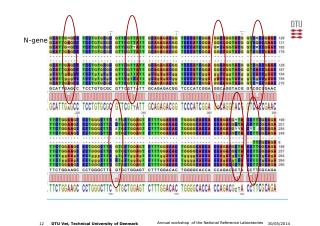
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Recommendations for next year

- Please assemble your sequences instead of sending several sequences for one isolate
- Request for chromatograms for further analysis
- Request for further information about sequencing methods

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