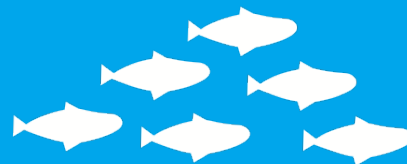




Veterinærinstituttet
Norwegian Veterinary Institute

Helgenomsekvensering av ILAV

Bjørn Spilsberg, Norwegian Veterinary Institute



Høykapasitets sekvensering

High throughput sequencing

- Vanlig sekvensering = Sanger sekvensering
- HTS - «High throughput sequencing»
- NGS - «Next Generation Sequencing»
- WGS - «Whole Genome Sequencing»
- Sanger sekvensering og HTS gir samme type data
- HTS gir veldig mye mer data
- Samtidig er kostnaden omtrent lik
- ”Gamechanger”

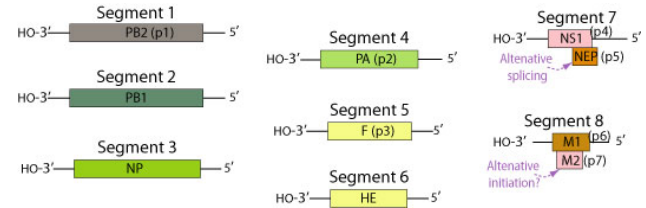


Hvorfor?

- Primært for å gjøre bedre slektskapsanalyser (10x mer data å bygge på)
- WGS gir mer informasjon og dermed sikrere analyser enn Sanger sekvensering
- Sammenligning av hele genomet og de enkelte segmentene kan gi ekstra informasjon

Sammenligning sanger sekvensering og amplikon sekvensering

- Glesvær genom 13 227 basepar
- Sanger sekvensering
 - Segment 6: 868 basepar
 - segment 5: 779 basepar
- Amplikon sekvensering: alle 8 segmenter: 12 287 basepar - 92,9%



Status

- Selve sekvenserings metoden er verifisert, publisert og klar til bruk
- Bioinformatikk: programmer og arbeidsflyt er identifisert
- Men det mangler en automatisk applikasjon (pipeline)
- Per idag kan metoden brukes til prosjekter og forskning
- Det er usikkert når vi kan få finansiering til å utvikle en pipeline for å bruke metoden i rutineanalyser

 frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 30 May 2024
DOI 10.3389/fmicb.2024.1392607



OPEN ACCESS

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RECEIVED 27 February 2024
ACCEPTED 07 May 2024
PUBLISHED 30 May 2024

CITATION
Spilberg B, Leithaug M, Christiansen DH,
Dahl MM, Petersen PE, Lagesen K,
Fiskebeck EMLZ, Moldal T and Boye M (2024)
Development and application of a whole
genome amplicon sequencing method for
infectious salmon anemia virus (ISAV).
Front. Microbiol. 15:1392607.
doi: 10.3389/fmicb.2024.1392607

Development and application of a whole genome amplicon sequencing method for infectious salmon anemia virus (ISAV)

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Genetisk distanse

- SNP = Single Nucleotid Polymorphism = mutasjon
- Hvor likt trenger to sekvenser være for å være like?
- Hvor ulik trenger to sekvenser være for å være ulike?

```
AGGTTATGTCGACAACATTAAGGAGGAGACA
| | | | | | | | | | | | | | x | | | | | | | | | | x | | | |
AGGTTATGTCGACAAGATTAAGGAGGTGACA
```

Distanse = 2

Primer design

CTTGTGGACGAACARACGC

a PRIMAL SCHEME

Scheme name

Scheme name

Email

Fasta
 Choose file: No file chosen

One or more viral reference genomes in FASTA format

Amplicon length

Overlap

[Generate my scheme!](#)

Want to try it out but no genomes to hand?
[Download CHIKV_demo.fasta](#)

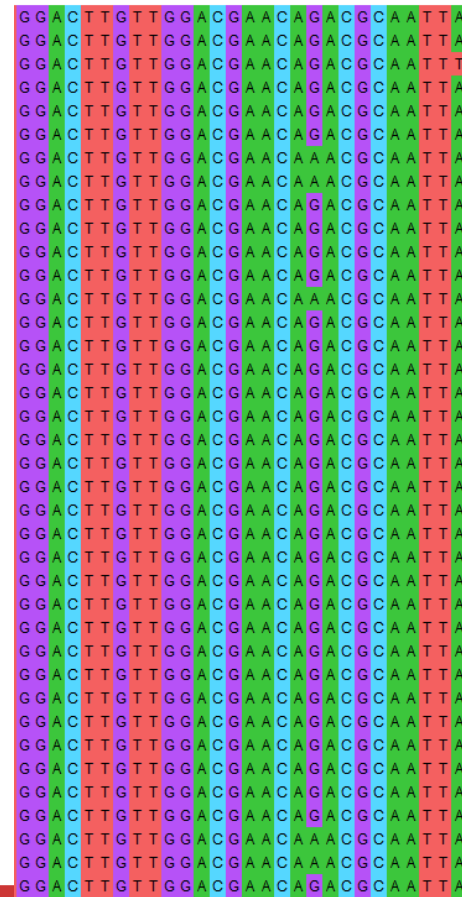
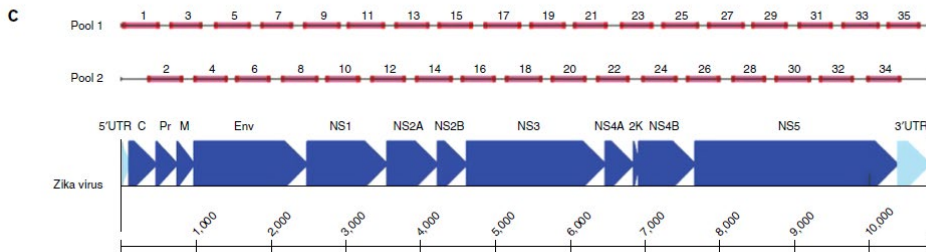
b

Job Name: CHIKV
 FASTA file: uploads/2018/11/09/CHIKV_demo.fasta
 Amplicon length: 400
 Overlap: 75

[Download as CSV](#)

[Primer Table](#)

Region No.	Pool	Left Primer Name	Left Primer Sequence	Right Primer Name	Right Primer Sequence
1	1	400_1_LEFT_3	CTTTTAAAGGCGCTCGAAGT	400_3_RIGHT_3	ACGGCCATCACCTCTGTAGT
2	2	400_2_LEFT_1	TGTCGACAGAGACTACTG	400_2_RIGHT_1	CGGCCCTTGTGSACTATGA
3	1	400_3_LEFT_1	GTNCTGGGAGGTTGACACA	400_3_RIGHT_1	TAAAGGCTGSGGCTATCCTTA
4	2	400_4_LEFT_4	TGGTGTTCACATTAAGGGCA	400_4_RIGHT_4	CTTCAATGTTGTCTGTTGGT
5	1	400_5_LEFT_2	TGTGATCAAAFFACGGCAGTC	400_5_RIGHT_2	GTCTCAAGCGGATTCACACG
6	2	400_6_LEFT_6	ACAGAGGGCTCATACCCATTC	400_6_RIGHT_6	GGCCCTCTGSAAGTCTCATTA
7	1	400_7_LEFT_1	TACAGGACGACAGGAGATGT	400_7_RIGHT_1	TTGTACCACTGGTGGCTTT
8	2	400_8_LEFT_3	GGAGCAGTGGAGACTGTGAC	400_8_RIGHT_3	GGACAGCGGGGGCAATTTTA
9	1	400_9_LEFT_3	TSCAAGAAAGAGACTCCAG	400_9_RIGHT_3	GGCAATTAAGCTCCAGATGG
10	2	400_10_LEFT_0	ATCTGACGCTGGCTGATTCG	400_10_RIGHT_0	GGTTTCTTGAAGCCGCTGCT



Joshua Quick, Nathan D Grubaugh, Steven T Pullan, Ingra M Claro, Andrew D Smith, Karthik Gangavarapu, Glenn Oliveira, Refugio Robles-Sikisaka, Thomas F Rogers, Nathan A Beutler, Dennis R Burton, Lia Laura Lewis-Ximenez, Jaqueline Goes de Jesus, Marta Giovanetti, Sarah C Hill, Allison Black, Trevor Bedford, Miles W Carroll, Marcio Nunes, Luiz Carlos Alcantara Jr., Ester C Sabino, Sally A Baylis, Nuno R Faria, Matthew Loose, Jared T Simpson, Oliver G Pybus, Kristian G Andersen & Nicholas J Loman

Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples
 Nature Protocols volume 12, pages1261–1276 (2017)

Sequencing accuracy

TABLE 1 Comparison of Sanger sequencing and amplicon sequencing of Glesvær samples (distance* (%SNPs/site)/numbers of SNPs).

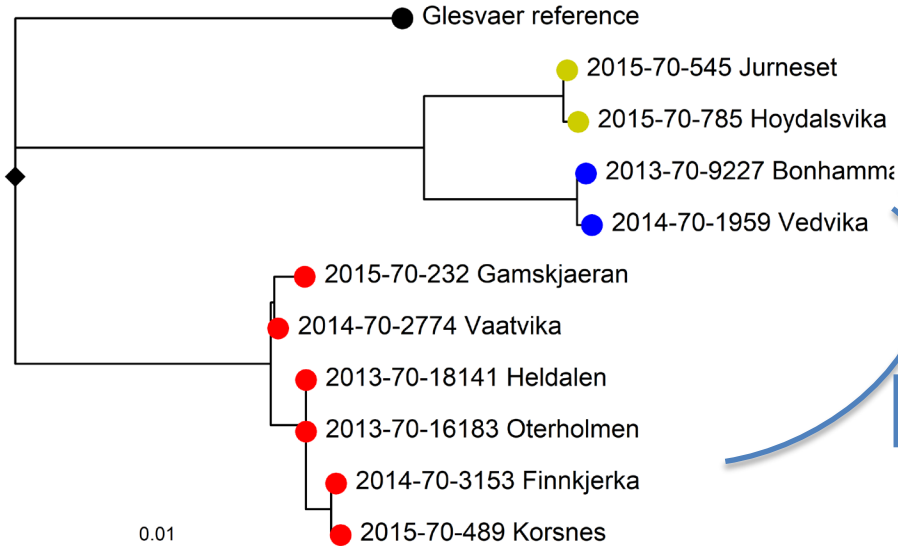
Reference or sample	Sample source	Technology	1	2	3	4	5	6	7
1. Glesvær (Merour et al., 2011)	CCS	Sanger							
2. Glesvær (Markussen et al., 2008)	CCS	Sanger	0,0743/9						
3. Glesvær-Tissue-1	Tissue	HTS amplicon	0,0407/5	0,1146/14					
4. Glesvær-Tissue-2	Tissue	HTS amplicon	0,0329/4	0,1067/13	0,0083/1				
5. Glesvær-CCS-1	CCS	HTS amplicon	0,0169/2	0,0907/11	0,0247/3	0,0169/2			
6. Glesvær-CCS-2	CCS	HTS amplicon	0,0169/2	0,0907/11	0,0247/3	0,0169/2	0/0		
7. Glesvær-CCS-3	CCS	HTS amplicon	0,0169/2	0,0907/11	0,0247/3	0,0169/2	0,0004/0	0,0004/0	

* Patristic distance was extracted from a phylogenetic tree generated in IQtree with a transversion evolutionary model

Gjennomsnittlig distanse mellom sekvenserte
Glesvær prøver (amplicon sekvensering)
og Glesvær referanser (Sanger sekvensering): 0,00063

MPP: distance to Sanger genome ≤ 0.001

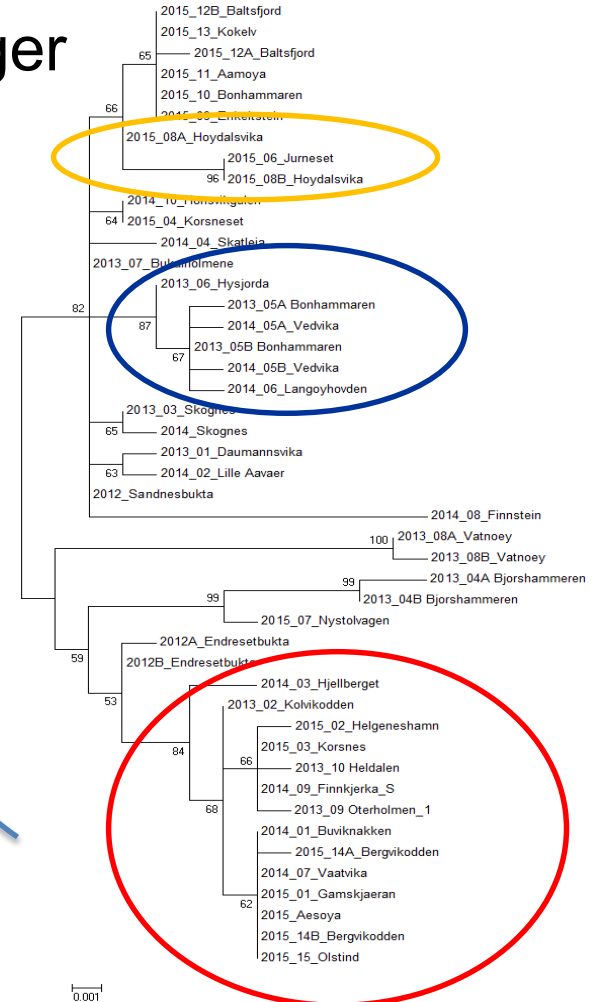
HTS



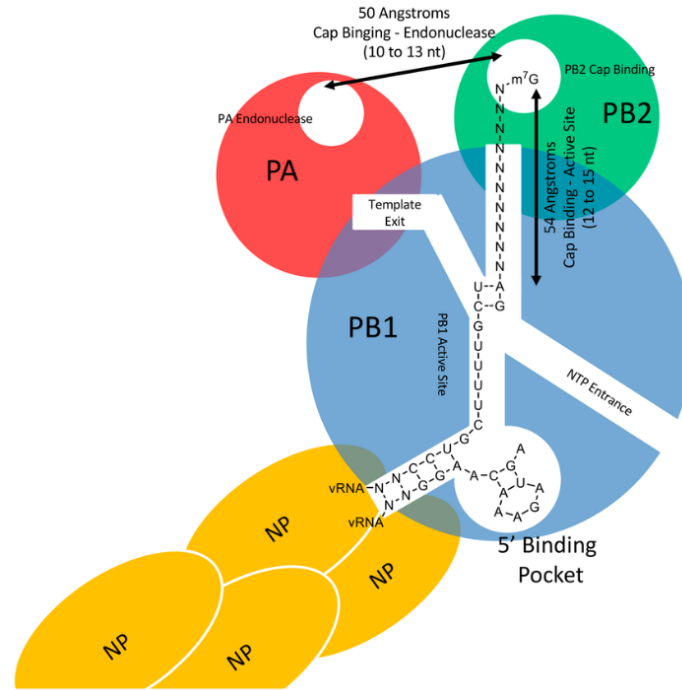
Sanger

61 - 5,5

153 - 10

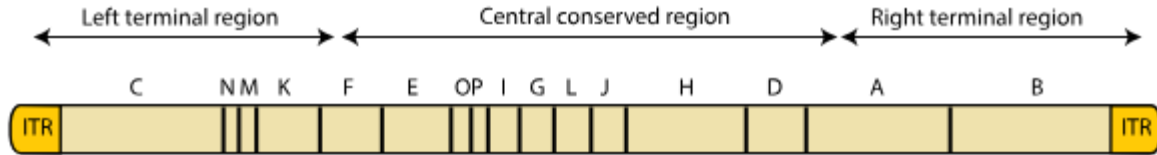
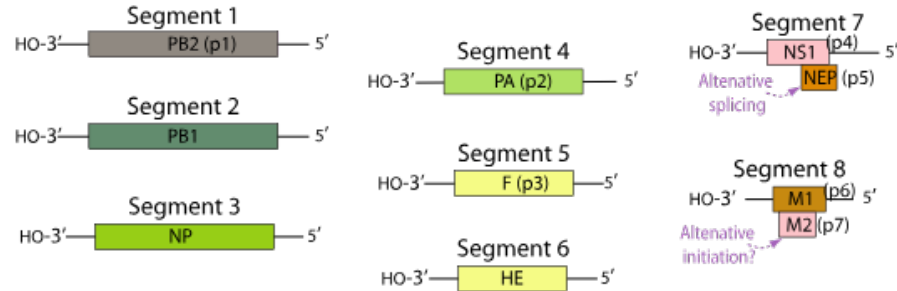


The ability of ISAV to adapt to a changing environment

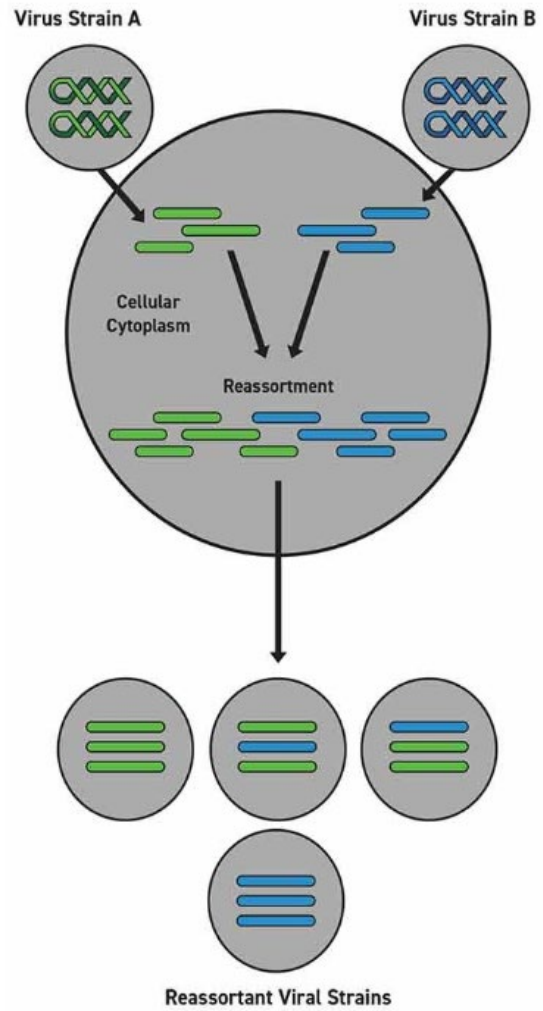


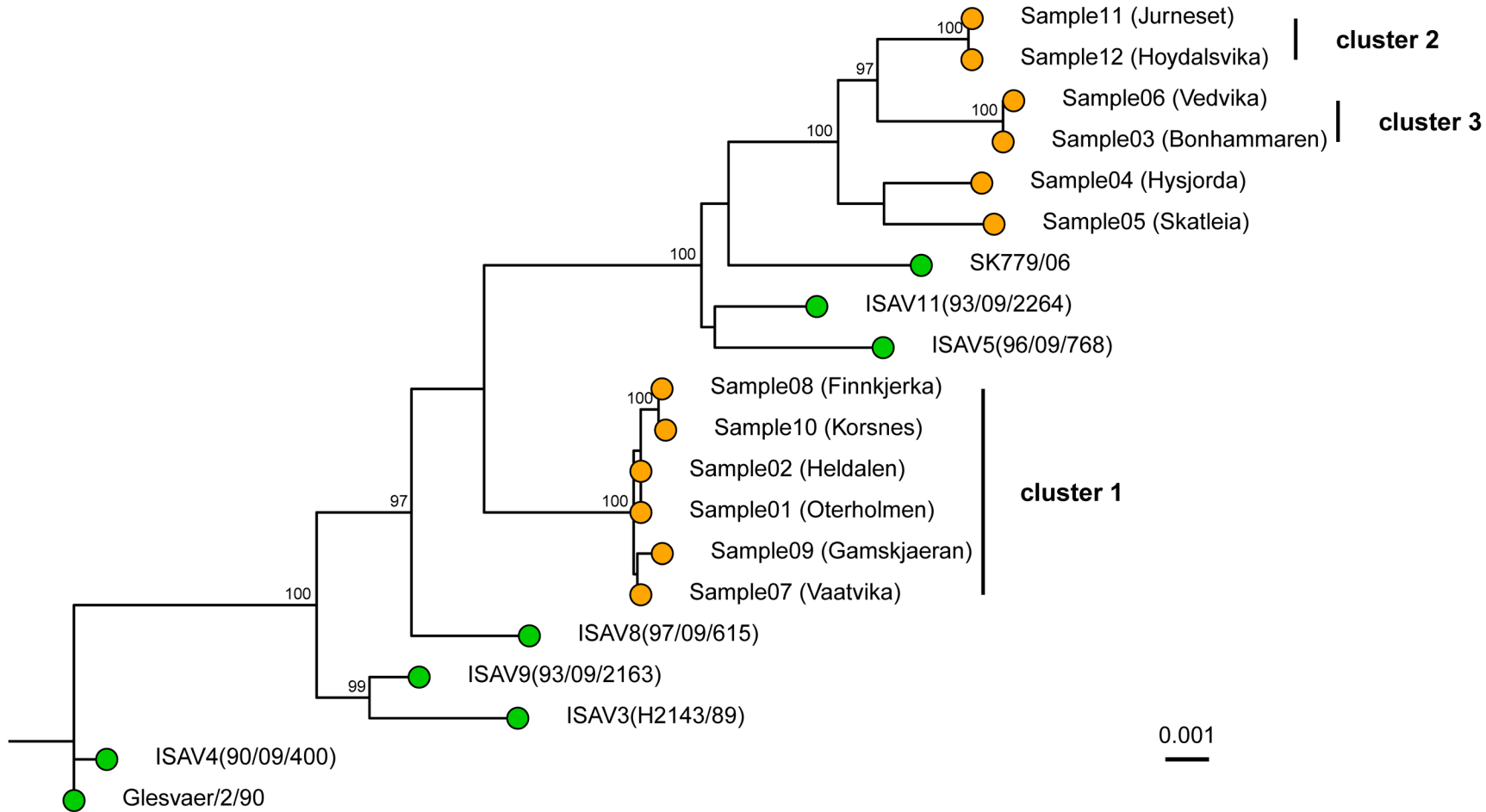
RNA-dependent RNA polymerase (RdRp)

The ability of ISAV to adapt to a changing environment



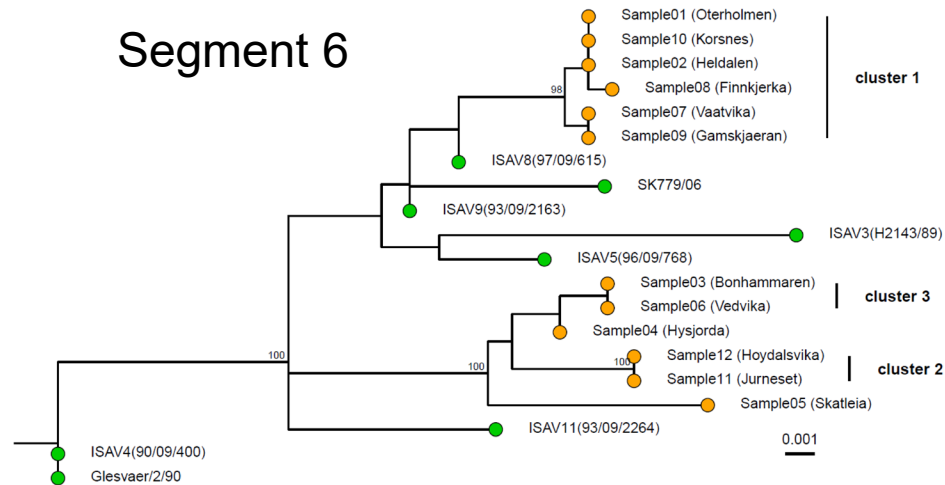
ITR= Inverted terminal repeats





(A)

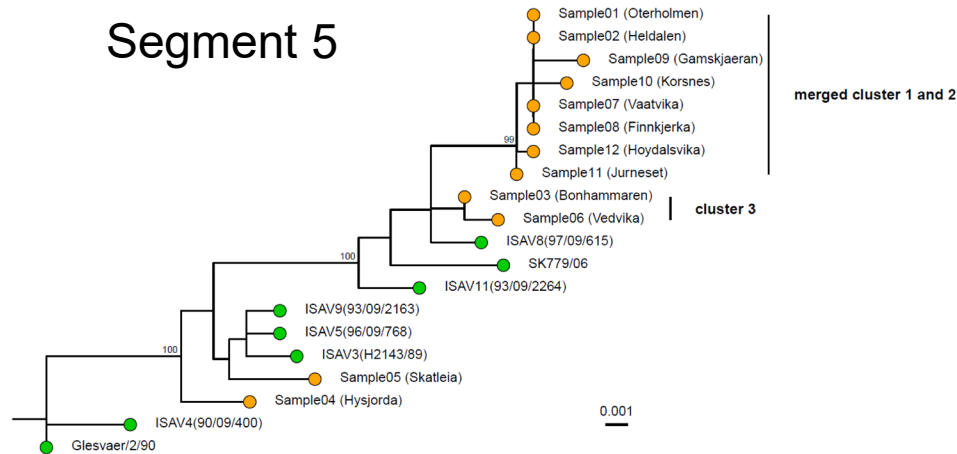
Segment 6



Where do sample 11/12 belong?

(B)

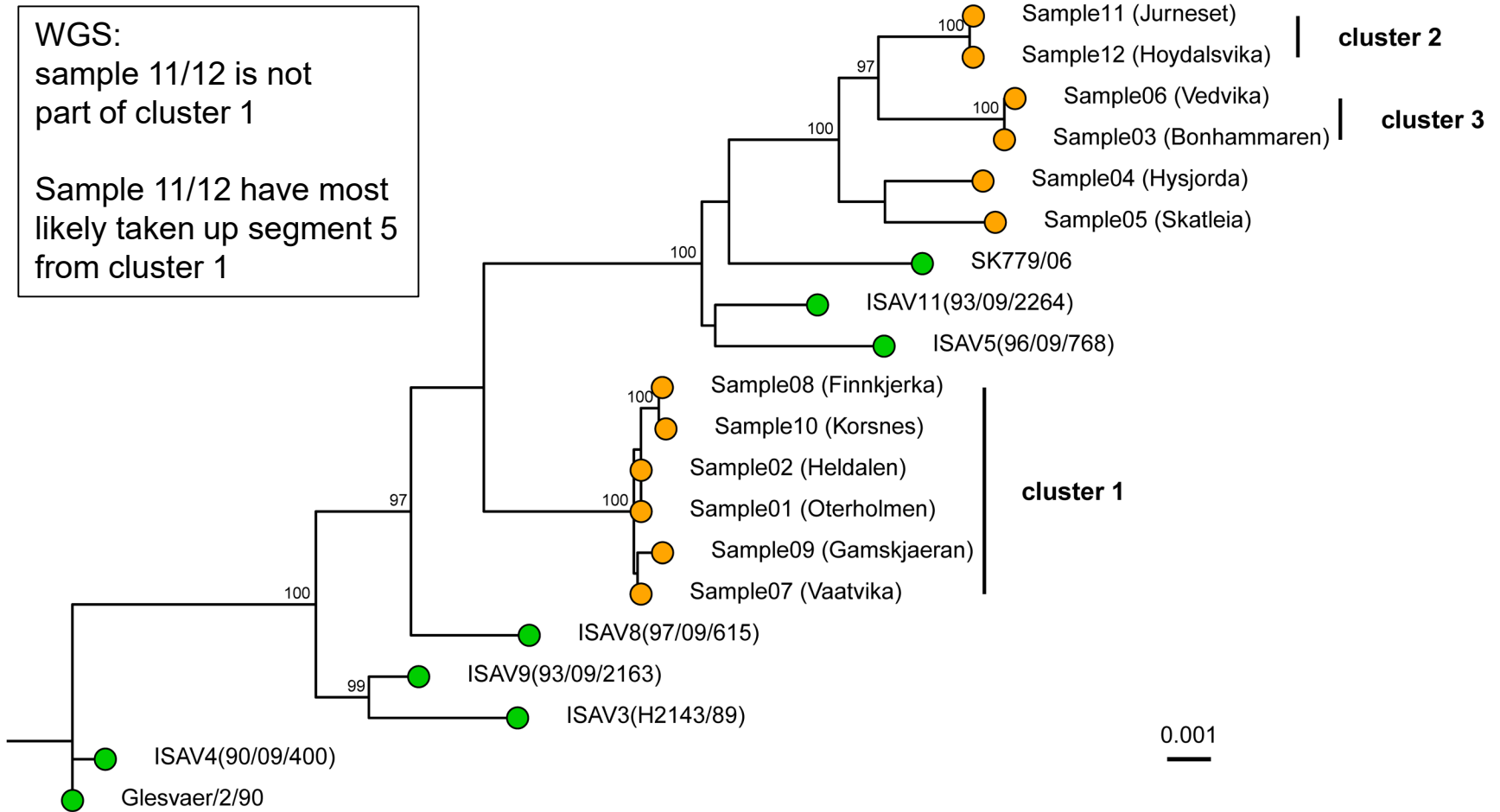
Segment 5



WGS:

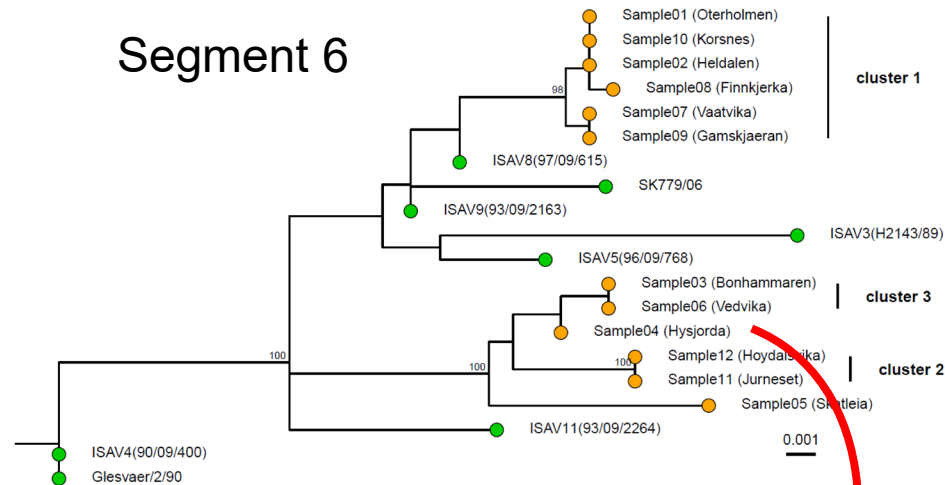
sample 11/12 is not
part of cluster 1

Sample 11/12 have most
likely taken up segment 5
from cluster 1



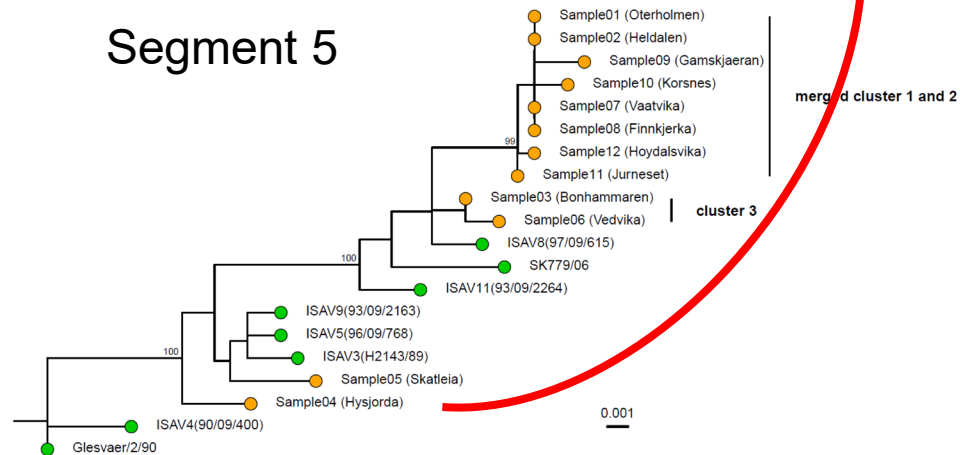
(A)

Segment 6



(B)

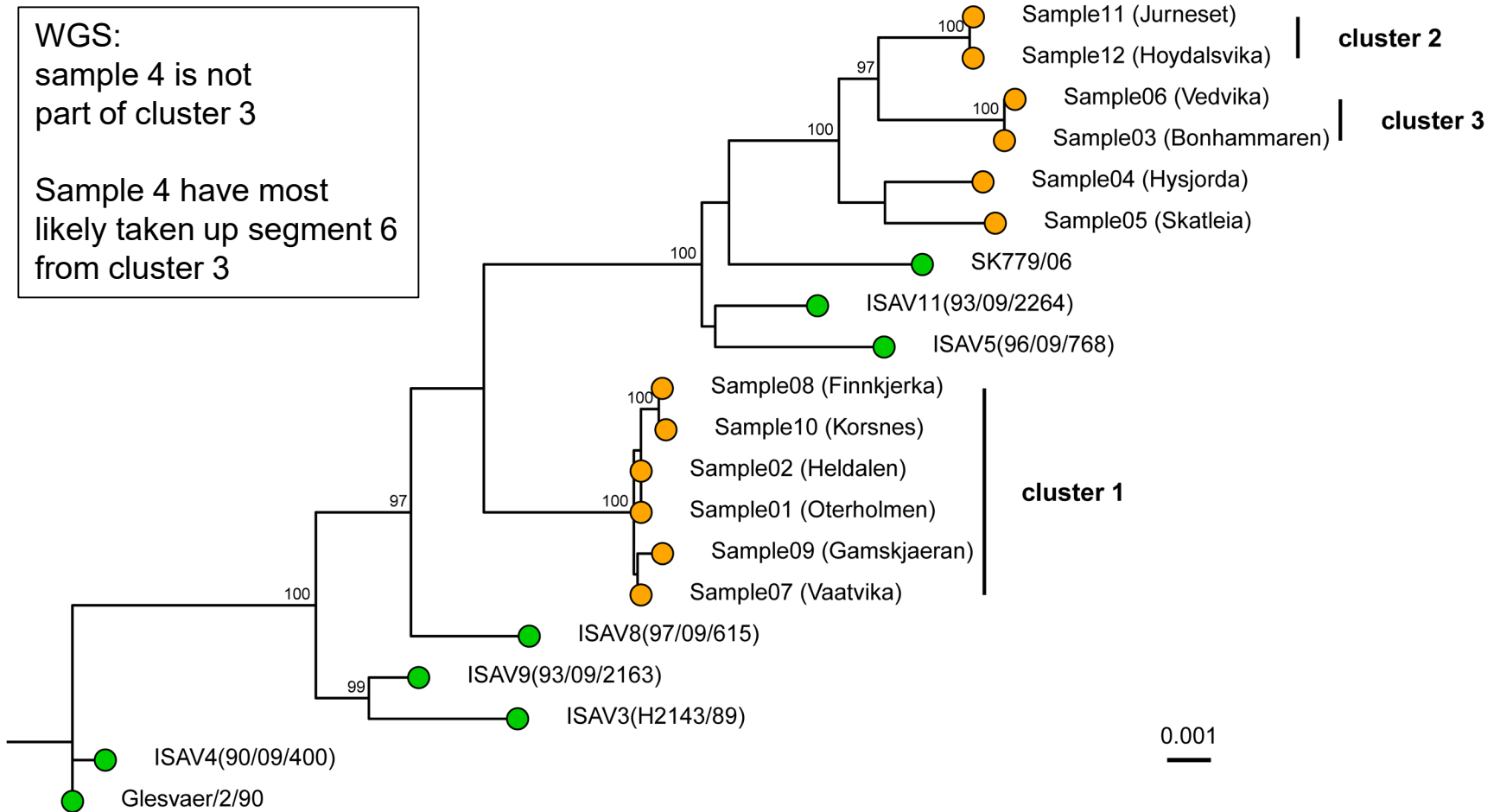
Segment 5



Where do sample 4 belong?

WGS:
sample 4 is not
part of cluster 3

Sample 4 have most
likely taken up segment 6
from cluster 3



Acknowledgements

- Magnus Leithaug
- Debes Hammershaimb Christiansen
- Maria Marjunardóttir Dahl
- Petra Elisabeth Petersen
- Karin Lagesen
- Eve M. L. Z Fiskebeck
- Torfinn Moldal
- Mette Boye
- Monika Hjortås

*Faglig ambisiøs, fremtidsrettet og
samspillende - for Én helse!*



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