Helgenomsekvensering av ILAV

Bjørn Spilsberg, Norwegian Veterinary Institute
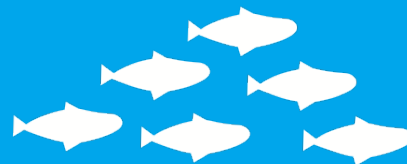
Høykapasitets sekvensering
High throughput sequencing

- 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%
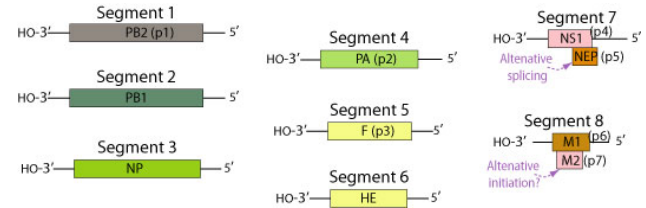
Status

- 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

Check for updates

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

Bjørn Spilsberg[1]*, Magnus Leithaug[1], Debes Hammershaimb Christiansen[2], Maria Marjunardóttir Dahl[2], Petra Elisabeth Petersen[2], Karin Lagesen[3], Eve M. L. Z. Fiskebeck[3], Torfinn Moldal[4] and Mette Boye[1]

[1]Department of Analysis and Diagnostics, Norwegian Veterinary Institute, Ås, Norway, [2]National Reference Laboratory for Fish and Animal Diseases, Faroese Food and Veterinary Authority, Torshavn, Faroe Islands, [3]Department of Animal Health and Food Safety, Norwegian Veterinary Institute, Ås, Norway, [4]Department of Aquatic Animal Health and Welfare, Norwegian Veterinary Institute, Ås, Norway

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

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)

8

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 (amplikon sekvensering)
og Glesvær referanser (Sanger sekvensering): 0,00063

MPP: distance to Sanger genome ≤ 0.001
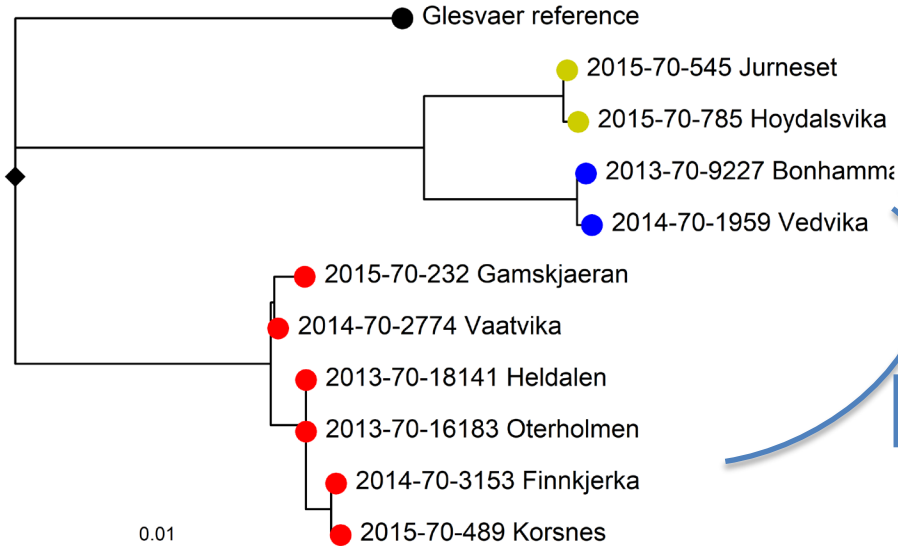
The ability of ISAV to adapt to a changing environment
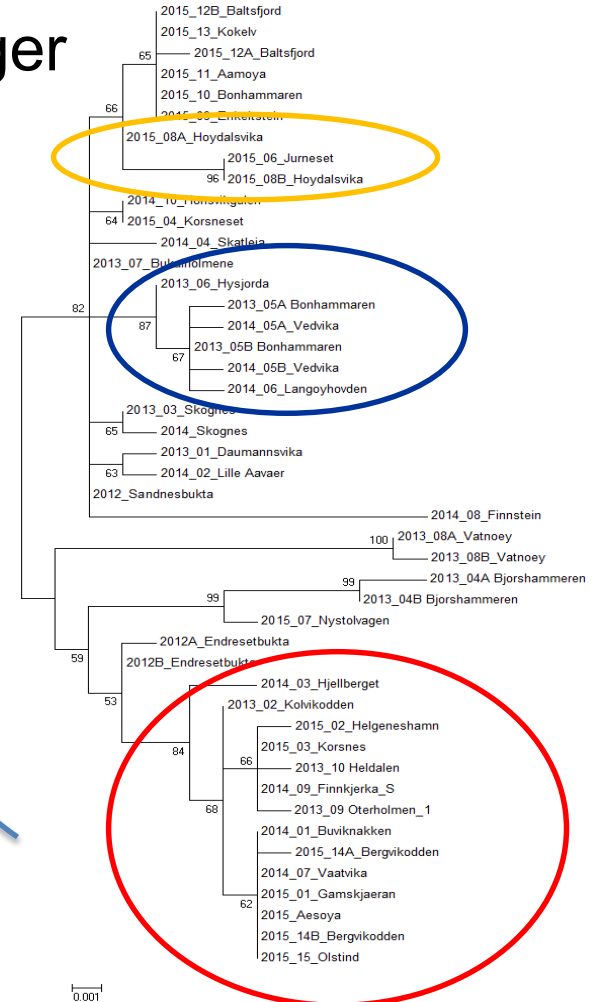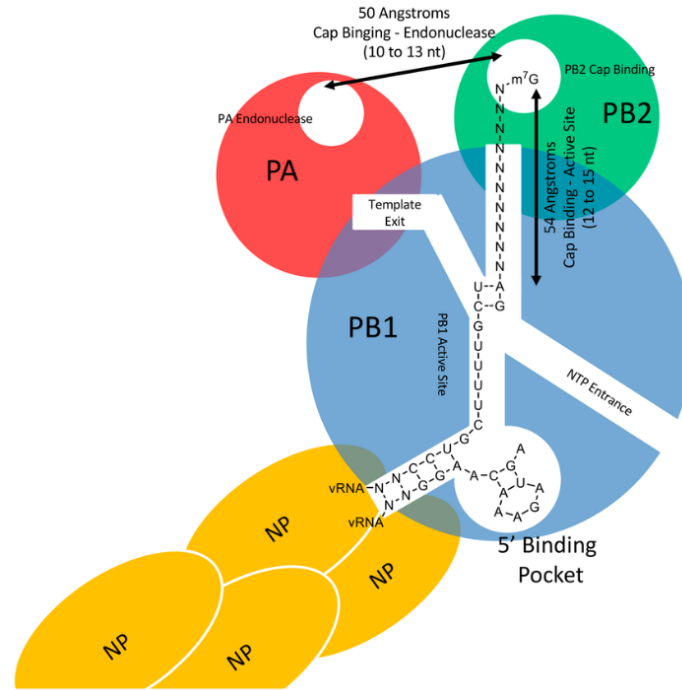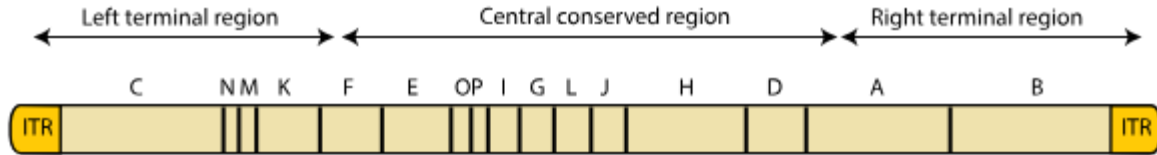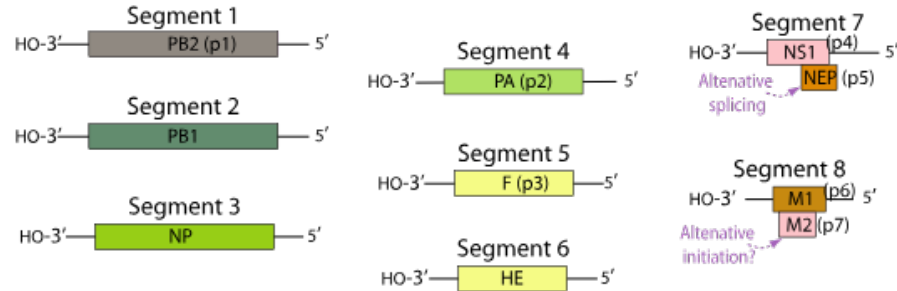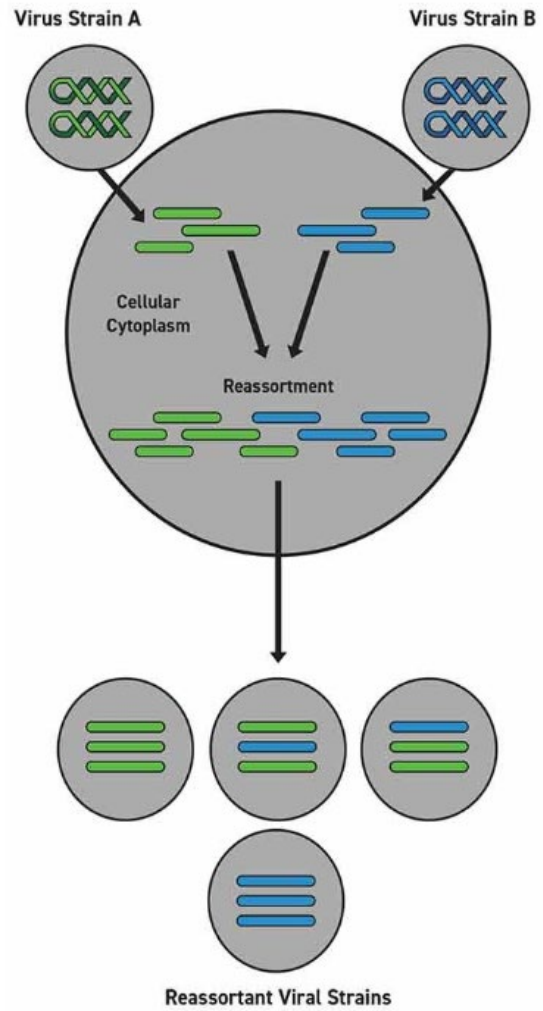

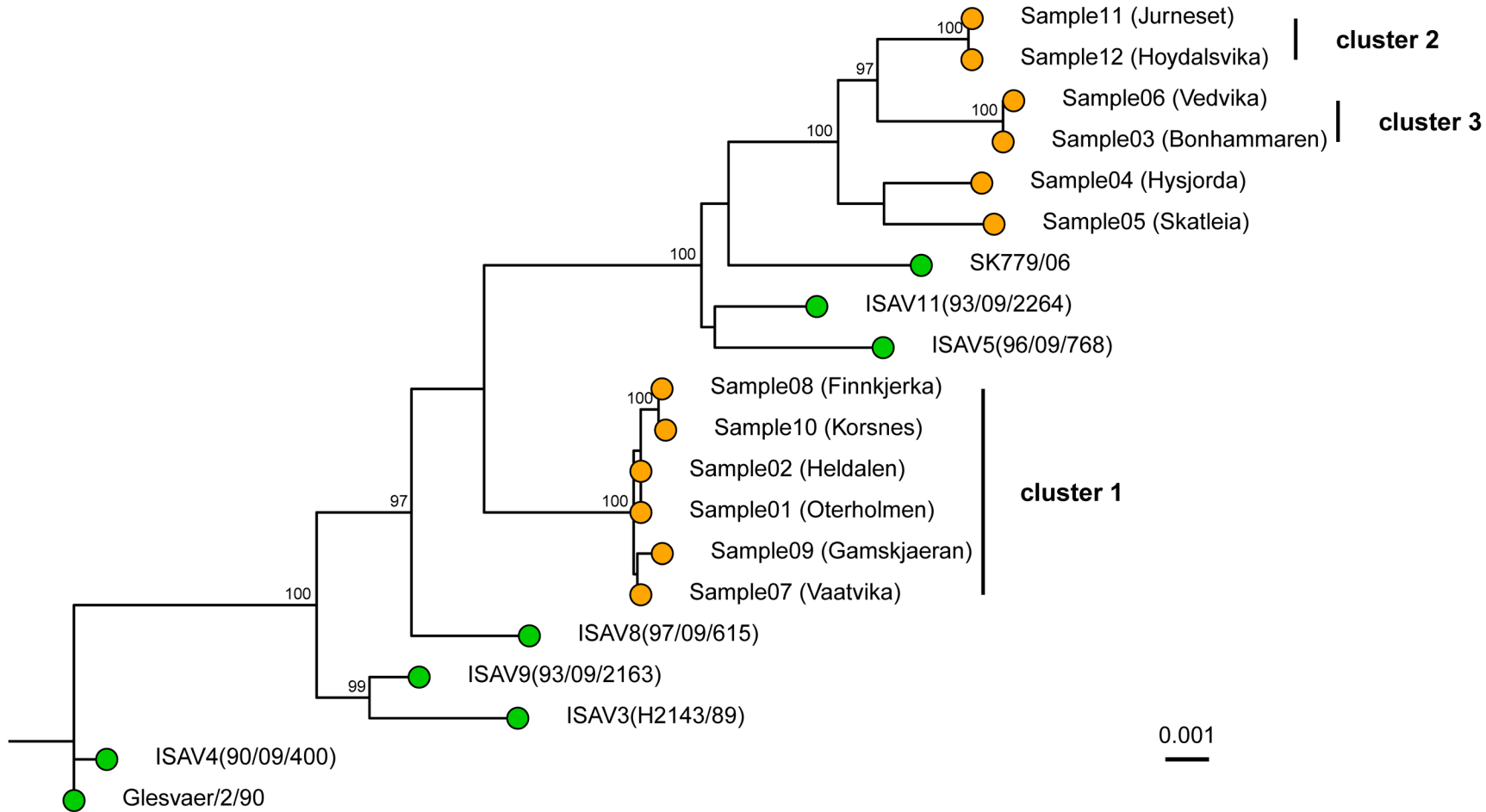RNA-dependent RNA polymerase (RdRp)

The ability of ISAV to adapt to a changing environment

ITR= Inverted terminal repeats

Virus Strain A Virus Strain B

Cellular Cytoplasm

Reassortment

Reassortant Viral Strains

(A) Segment 6

Sample01 (Oterholmen)
Sample10 (Korsnes)
Sample02 (Heldalen)
Sample08 (Finnkjerka)
Sample07 (Vaatvika)
Sample09 (Gamskjaeran)
cluster 1
98
ISAV8(97/09/615)
SK779/06
ISAV9(93/09/2163)
ISAV3(H2143/89)
ISAV5(96/09/768)
Sample03 (Bonhammaren)
Sample06 (Vedvika)
cluster 3
Sample04 (Hysjorda)
Sample12 (Hoydalsvika)
Sample11 (Jurneset)
cluster 2
100
100
100
Sample05 (Skatleia)
ISAV11(93/09/2264)
0.001
ISAV4(90/09/400)
Glesvaer/2/90

Where do sample 11/12 belong?

(B) Segment 5

Sample01 (Oterholmen)
Sample02 (Heldalen)
Sample09 (Gamskjaeran)
Sample10 (Korsnes)
Sample07 (Vaatvika)
Sample08 (Finnkjerka)
Sample12 (Hoydalsvika)
Sample11 (Jurneset)
merged cluster 1 and 2
99
Sample03 (Bonhammaren)
Sample06 (Vedvika)
cluster 3
ISAV8(97/09/615)
SK779/06
100
ISAV11(93/09/2264)
ISAV9(93/09/2163)
ISAV5(96/09/768)
ISAV3(H2143/89)
100
Sample05 (Skatleia)
Sample04 (Hysjorda)
0.001
ISAV4(90/09/400)
Glesvaer/2/90

15

(A)

Segment 6

Sample01 (Oterholmen)
Sample10 (Korsnes)
Sample02 (Heldalen)
Sample08 (Finnkjerka)
Sample07 (Vaatvika)
Sample09 (Gamskjaeran)
cluster 1
98
ISAV8(97/09/615)
SK779/06
ISAV9(93/09/2163)
ISAV3(H2143/89)
ISAV5(96/09/768)
Sample03 (Bonhammaren)
Sample06 (Vedvika)
cluster 3
Sample04 (Hysjorda)
Sample12 (Hoydalsvika)
Sample11 (Jurneset)
cluster 2
100
100
100
Sample05 (Skatleia)
ISAV11(93/09/2264)
ISAV4(90/09/400)
Glesvaer/2/90
0.001

(B)

Segment 5

Sample01 (Oterholmen)
Sample02 (Heldalen)
Sample09 (Gamskjaeran)
Sample10 (Korsnes)
Sample07 (Vaatvika)
Sample08 (Finnkjerka)
Sample12 (Hoydalsvika)
Sample11 (Jurneset)
merged cluster 1 and 2
99
Sample03 (Bonhammaren)
Sample06 (Vedvika)
cluster 3
ISAV8(97/09/615)
SK779/06
ISAV11(93/09/2264)
100
ISAV9(93/09/2163)
ISAV5(96/09/768)
ISAV3(H2143/89)
100
Sample05 (Skatleia)
Sample04 (Hysjorda)
ISAV4(90/09/400)
Glesvaer/2/90
0.001

Where do sample 4 belong?
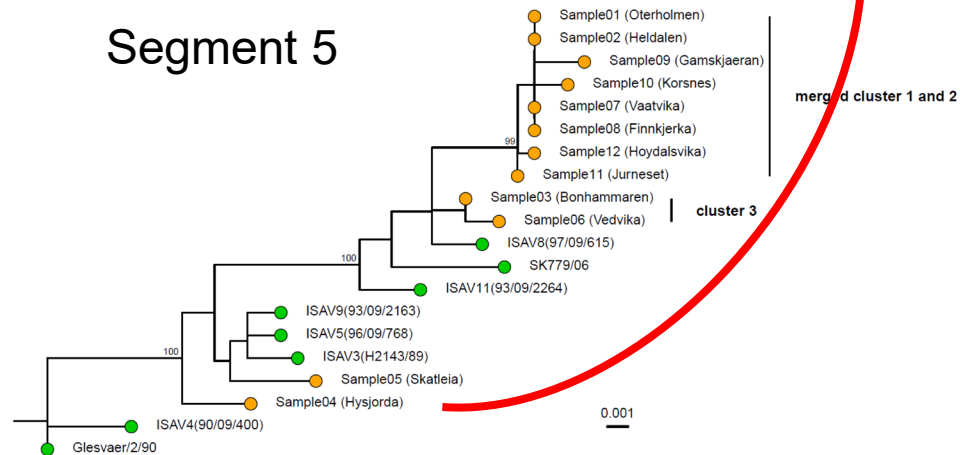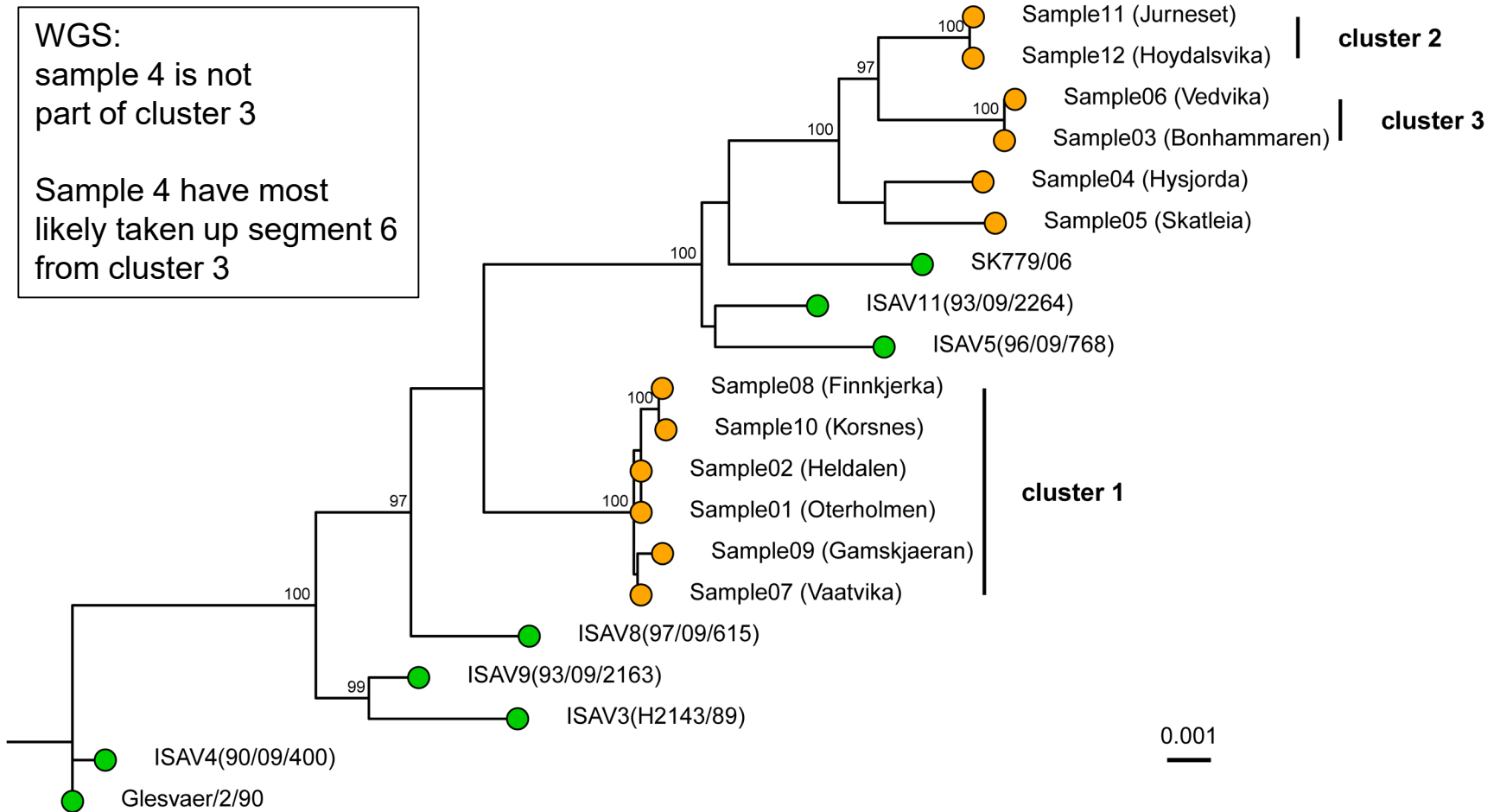
17

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