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AQUAVAC®

ID: 2018-ms-0978

Molecular studies of SAV3 isolates from mild and severe PD outbreaks

PD TriNation meeting,

Bergen, Norway

13-15th Mar 2018

Ingunn Sommerset

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Project background

- The majority of farms in the SAV3 endemic zone vaccinate against PD
- Although specific PD mortality has decreased the last years, substantial *differences* between and within farms are registered
- Biological factors which may influence the outcome of an infection in vaccinated stocks:
  - Infectious pressure
  - History/origin of the smolt (smolt quality)
  - Stressful events
  - Other diseases e.g. gill health problems
- «Hot spot» locations/areas for recurring PD problems
- Can differences in PD mortality be (partly) explained by difference in SAV3 virulence?

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Project background

- **Karlsen et al. 2006:** 20 Norwegian isolates from 1997-2004: 0.66% nucleotide divergence in 1121 nt long strech, partial C-E3-E2 encoding sequences.
- **Jansen et al. 2010:** 33 SAV3 isolates from Aug 2006 – Oct 2009: 0 – 1.1% nucleotide divergence in the *451 nt* long stretch of the E2 gene → Homogeneous isolates despite large geographical distances
- **Petterson et al 2013**: 9 SAV3 isolates from 2009-2010: mean nucleotide divergence of 0.11% (0.04 – 0.22%) in *entire virus genome (11756 nt)* → High degree of sequence identity
  - Highest in E2 and 6K
  - Lowest in Capsid and nsP4 and nsP2
- **Merour et al. 2013**: A single aa substitution in E2 (A8V) is responsible for loss of virulence in sleepding disease (FW SAV2)

Molecular studies of SAV3 isolates from mild and severe PD outbreaks

- Project period: 2015 – 2017

- Main objectives:
  - Investigate if cases with high PD mortality could be attributed to recurring SAV3 gene (amino acid) sequences

  - If candidate “high virulent” and “low virulent” field isolates were identified from sequence data, perform controlled tank experiment to verify different pathology

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Project challenges

- No high PD mortality outbreak at MH sites in the project period → historical samples needed
- Major problems with PCR-sequencing of the selected genes → novel approach in design of primers

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Preliminary sequencing results:

Sample	CAP	P2	P3	E2
106	parts completed	no PCR/ sequencing results	no PCR/ sequencing results	no PCR/ sequencing results
114	complete	complete	parts completed	parts completed
129	complete	complete	parts completed	parts completed
134	complete	complete	parts completed	parts completed
158	no PCR/ sequencing results	parts completed	parts completed	parts completed
165	complete	parts completed	parts completed	parts completed
180	complete	complete	parts completed	parts completed
191	complete	complete	parts completed	complete
202	no PCR/ sequencing results	parts completed	no PCR/ sequencing results	no PCR/ sequencing results

Sequencing
complete
parts completed
no PCR/ sequencing results

<u>Status May 2016</u>: only 1 out of 9 isolates with complete capsid, NSP2 and E2 gene sequence by customary cDNA synthesis and primer design (!)

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Re-start primer design and optimal cDNA synthesis

- RNA isolated from heart tissue using Fibrous tissue kit
  - Improved RNA yields

- New approach with PCR primers in areas with genomic deletions publised by Petterson et al 2013 og 2016
  - forward and revers primers placed in deletion hot spots → avoid amplification of trash RNA

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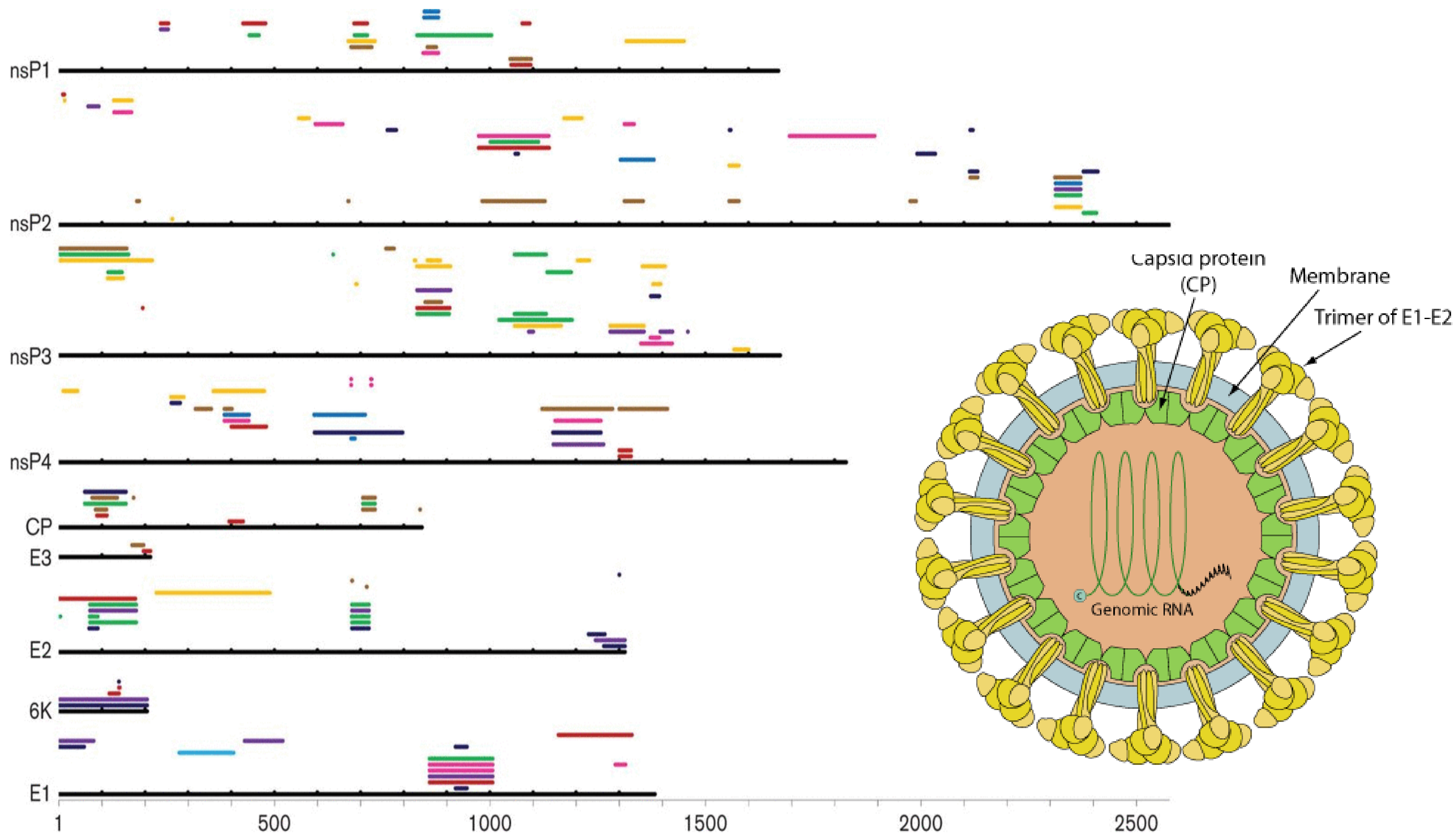

Fig 2. Distribution of genome deletions (colored strings) over the open reading frames in SAV3. From Petterson et al 2013. J Gen Virol 94: 1945-54

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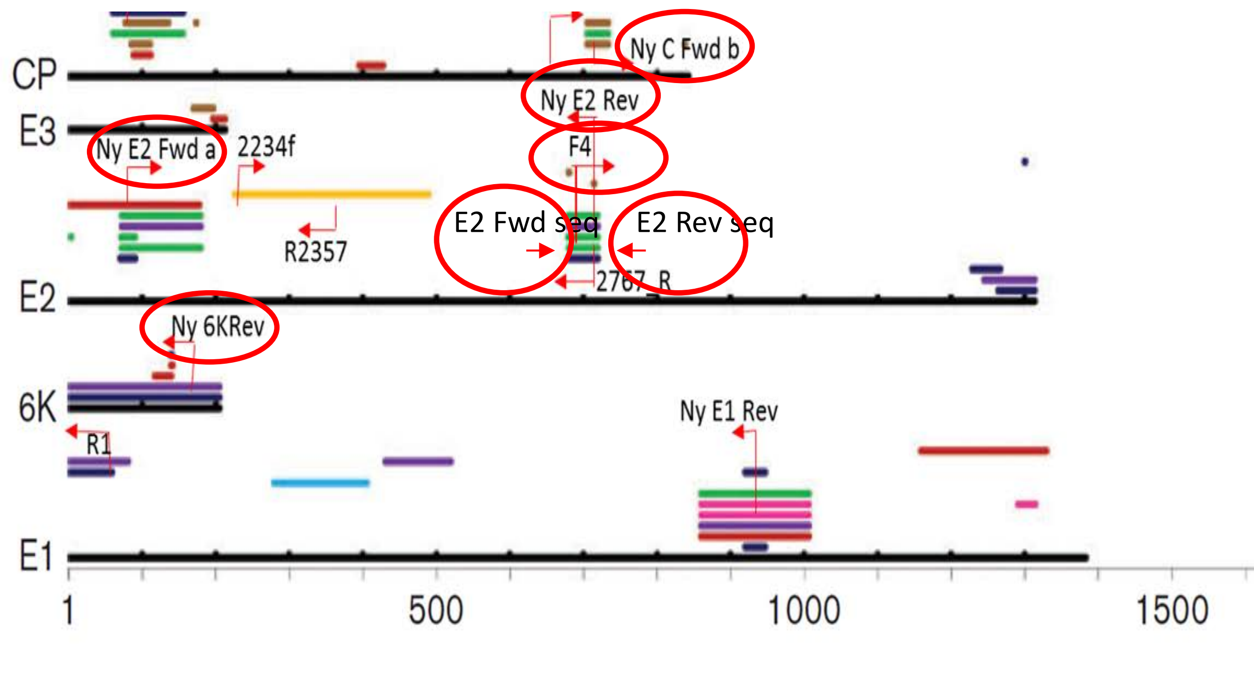
PCR and sequencing- primers



Located to deletion «hot spots» → avoid binding and amplification of trash RNA

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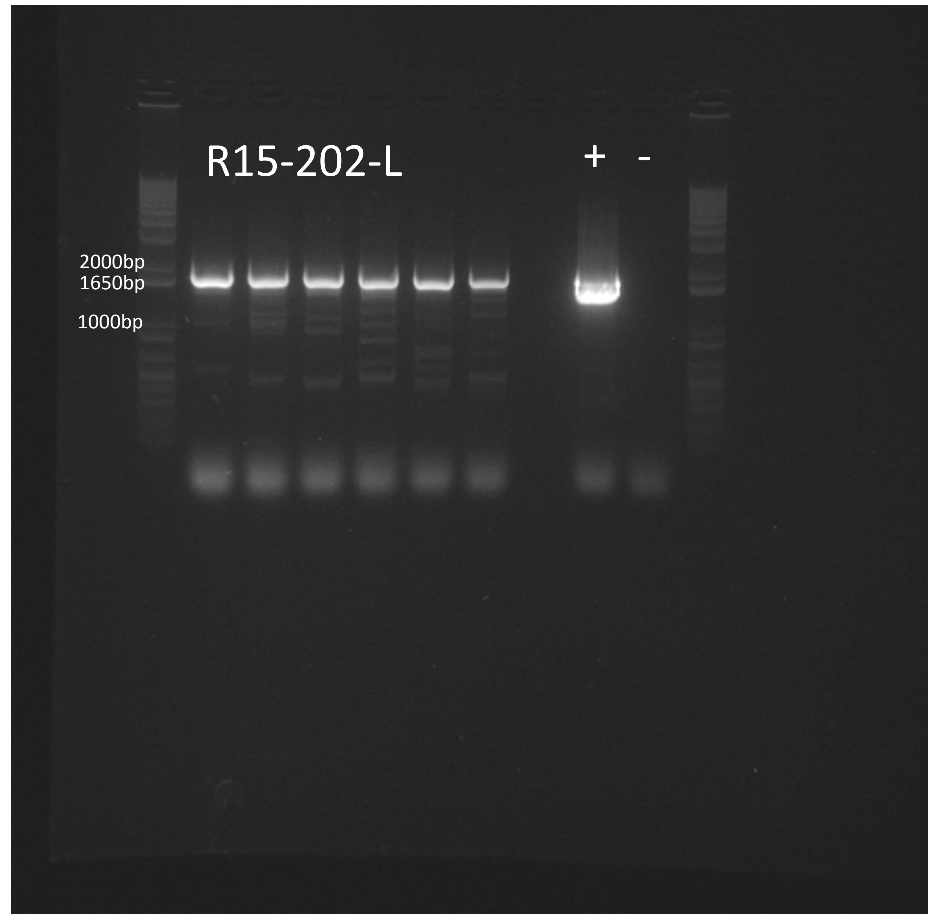
Example of PCR product for E3+E2

- One main band of 1700nt and several shorter products with deleted RNA seen on the gel
- Extraction and concentration of single 1700nt PCR product to achieve high quality chromatograms

ID 202 with Ct of 30



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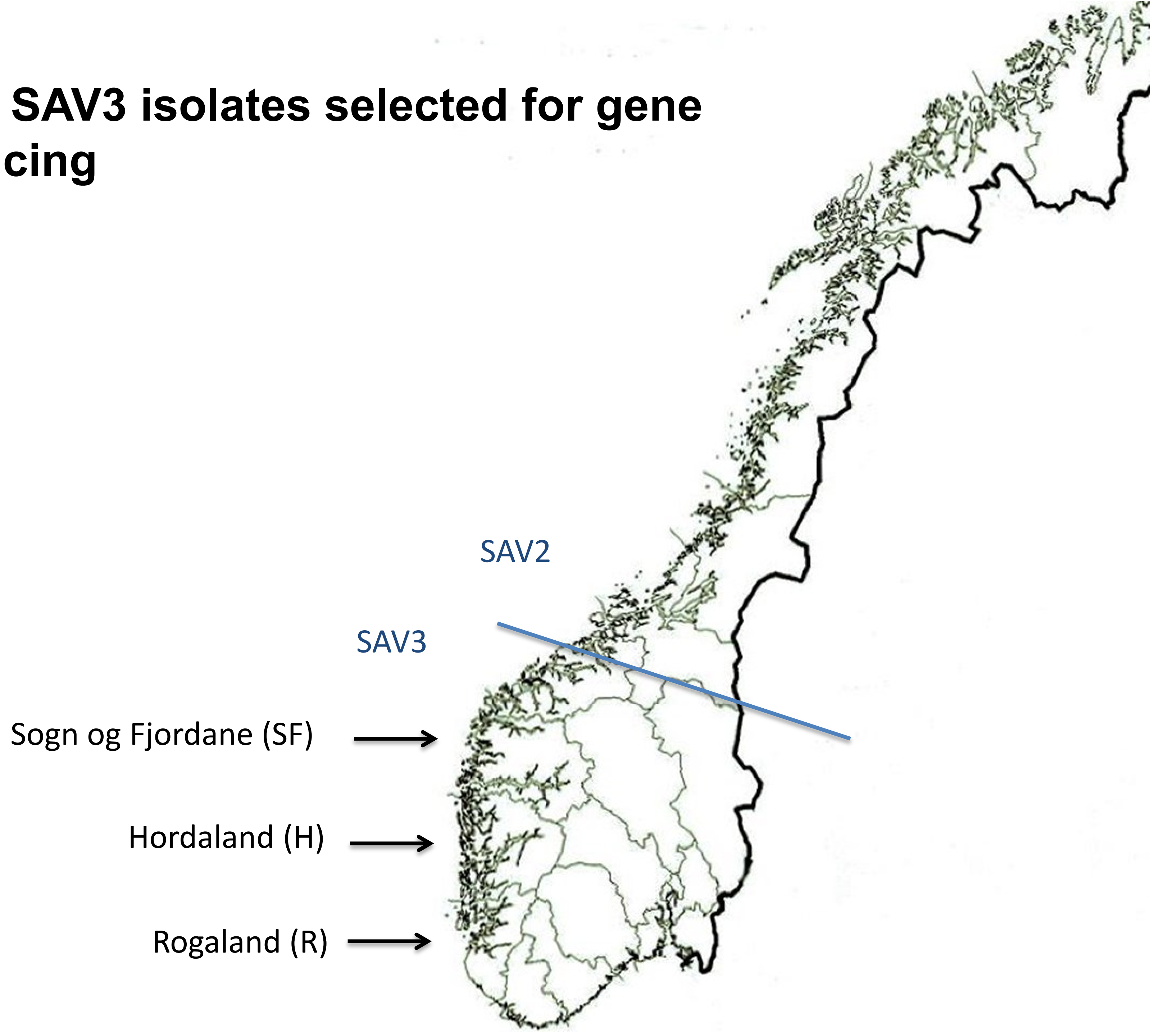
11 field SAV3 isolates selected for gene sequencing



11 field isolates selected for E2/E3 and capsid gene sequencing

No	Sample ID	County	Year	Mortality (H/L)
1	SF15-180-L	Sogn og Fjordane	2015	<5% (L)
2	SF15-191-L	Sogn og Fjordane	2015	3% (L)
3	SF16-229-L	Sogn og Fjordane	2016	1.5% (L)
4	H15-165-L	Hordaland	2015	< 5% (L)
5	R15-114 -L	Rogaland	2015	< 5% (L)
6	R15-202-L*	Rogaland	2015	< 5% (L)
7	R15-134-H*	Rogaland	2015	> 20% (H)
8	H16-244-H	Hordaland	2016	8-15% (H)
9	R10-V02-H	Rogaland	2011	> 20% (H)
10	R11-V04-H	Rogaland	2011	> 20% (H)
11	R12-V05-H	Rogaland	2012	> 20% (H)

*Neighbor farms

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Results E3+E2 gene: 1717 nt

Isolate	E3 87	E2 60	E2 561	E2 593	E2 597	E2 703	E2 864
SF15-180-L	C	T	T	A	T	G	G
SF15-191-L	C	C	T	A	T	G	G
SF16-229-L	C	T	T	A	T	G	G
H15-165-L	C	T	T	A	A	A	G
R15-114-L	C	T	T	A	T	G	G
R15-202-L*	C	T	T	A	T	G	A
R15-134-H*	C	T	T	A	T	G	A
H16-244-H	T/C dp	T	C	G	T	G	G
R10-V02-H	C	T	T	A	T	A	G
R11-V04-H	C	T	T	A	T	A	G
R12-V05-H	C	T	T	A	T	G	G

The table shows nucleotide positions in the E2 and E3 gene where differences are present between the 11 SAV3 isolates.

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Results translated E2 gene (protein): 438 aa

Isolate	E2 198	E2 199	E2 235
SF15-180-L	D	N	A
SF15-191-L	D	N	A
SF16-229-L	D	N	A
H15-165-L	D	K	T
R15-114-L	D	N	A
R15-202-L*	D	N	A
R15-134-H*	D	N	A
H16-244-H	G	N	A
R10-V02-H	D	N	T
R11-V04-H	D	N	T
R12-V05-H	D	N	A

Comparison - published sequence data:

Karlsen *et al* 2006 – no identical mutations

Jansen *et al* 2010 - no identical mutations

Petterson *et al* 2013 - no identical mutations

Merour *et al* 2013 – no identical mutations

Comparison - field mortality:

No obvious relationship between mortality and heterogenous amino acids in E2

The table shows aa positions in E2 where differences are present between the 11 SAV3 isolates.

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Results Capsid: 843 nt, 281 aa

Isolate	C 196	C 208	C 675
SF15-180-L	C	G	C
SF15-191-L	C	G	C
SF16-229-L	C/A dp	G	T
H15-165-L	C	G	C
R15-114-L	C	G	T
R15-202-L*	C	G	T
R15-134-H*	C	G	T
H16-244-H	C	A/G dp	C
R10-V02-H	C	G	C
R11-V04-H	C	G	C
R12-V05-H	C	G	T

Isolate	C 66
SF15-180-L	Q
SF15-191-L	Q
SF16-229-L	Q/K dp
H15-165-L	Q
R15-114-L	Q
R15-202-L*	Q
R15-134-H*	Q
H16-244-H	Q
R10-V02-H	Q
R11-V04-H	Q
R12-V05-H	Q

The blue table shows nucleotide- and the violet table aa positions where differences are present between the 11 SAV3 isolates.

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Summary:

- **Method** established for successful sequencing of complete E3+E2 and capsid gene
- **Very high sequence identity** despite different geographical regions, year of isolation and PD mortality for the 11 SAV3 isolates
  - E2 protein (438 aa): 3 aa differences
  - Capsid protein (281 aa): 1 aa difference
- No obvious correlation between sequence differences and mortality – thus **no evidence for «high» and «low» virulent SAV3 strains**

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