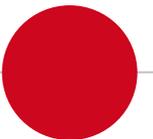


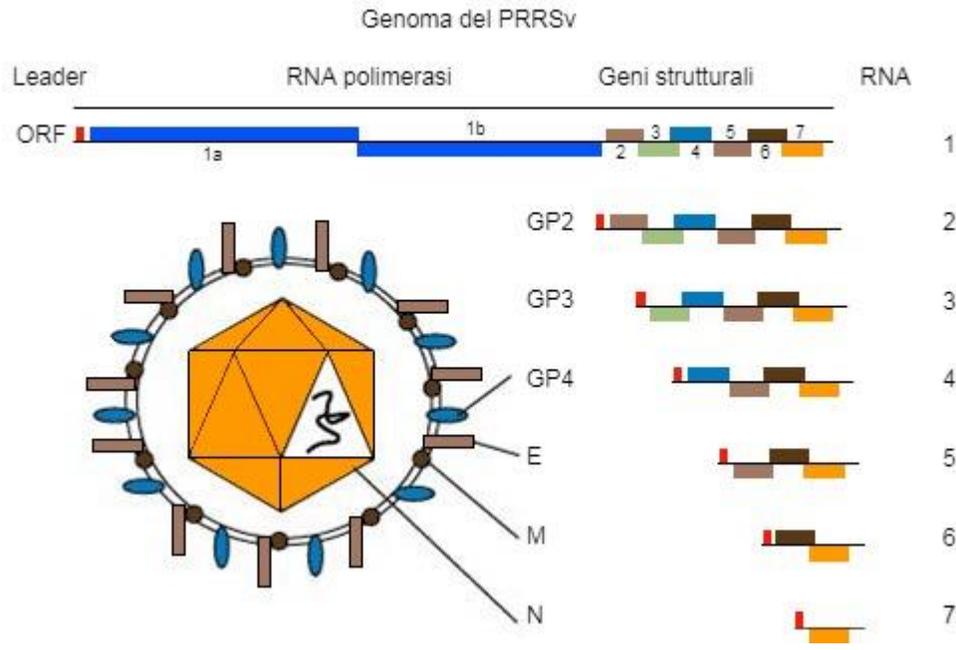
# Il sequenziamento genico come strumento complementare per il controllo della PRRS

**Denis Vio**

*Laboratorio di Patologia e Benessere Specie Suina  
Sezione Territoriale di Pordenone  
Istituto Zooprofilattico Sperimentale delle Venezie*



# ● Caratteristiche di PRRSV



- Genoma: RNA a filamento singolo

- Incline a mutazioni genetiche

- Selezione «naturale» dei ceppi

- Coesistenza di ceppi diversi

## ● Selezione «naturale» dei ceppi

- Fattori non dipendenti solo dal virus ma anche dall'ospite/ambiente

Capacità del sistema immunitaria del suino di far fronte alla replicazione (e alla trasmissione) del virus



Meno il virus replica → meno opportunità ha di mutare

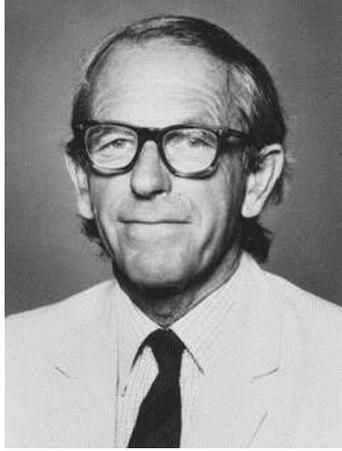


Biosicurezza (riduzione carica virale/meno replicazione)

- Lo stesso virus in situazioni diverse subisce un'evoluzione diversa

# ● Che cosa è il sequenziamento

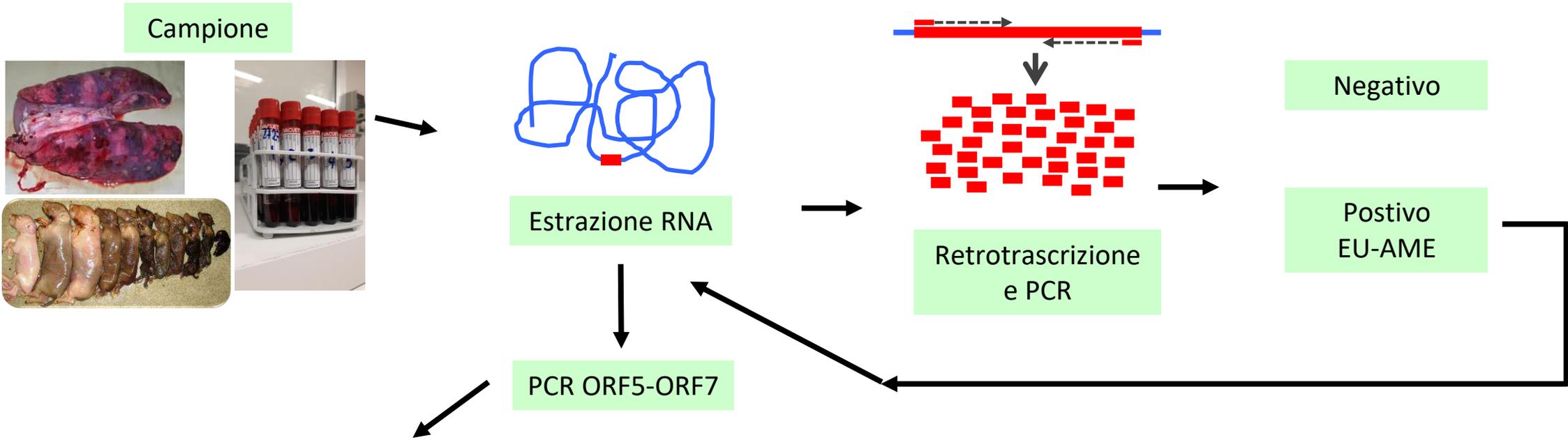
- Tecnica biomolecolare che permette di ricostruire l'ordine dei nucleotidi (A, C, G e T/U) che formano una molecola di DNA/RNA.
- Sequenziamento Sanger: utilizzato di routine, max 800-1000 pb, costi contenuti
- Pirosequenziamento: rapido e preciso ma applicabile a sequenze corte (max 300-500 pb)
- NGS (Next Generation Sequencing): intero genoma del campione in analisi



Frederick Sanger (1918-2013)  
Premio Nobel per la Chimica  
1958 e 1980



# Dal campione alla sequenza



Estrazione RNA

Retrotrascrizione e PCR

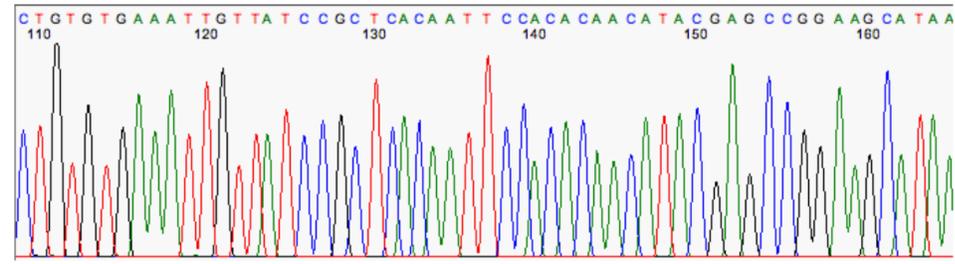
Negativo

Postivo EU-AME

PCR ORF5-ORF7



Sequenziamento



cromatogramma

sequenza

```
>Mg_MxT01_06C10
GACACAAGTTAACAAAATGACAGTGGTGTGTACGGGTGCGACGACAGCGT
CAATTGCTTGGATTCCAGATAAGACAGTAGTTCTTGGTGAAAATTTTACA
GTGAAATACAAAACAAACAGCGGACAAATACAACACTATCCCTTTTAAAGA
ACCGTCAACTAAGGATGACATATATTTGCTACAAGTAGACAGTCTCGCCC
CATCTACAAAGTACATTTACATTGGAATCCAAAAATGGTTTAGGACGA
GCTGAATCGAATGAGTACACATGCACAACACTATGGCTGCATCAGAGGAGTC
TGGAGTTAGTGGTCTCTAATAGGAGGAGTTGTATTGATATCAATTGCGC
TACTGCTAATCATAATAGTCATTGCGTTGATTTTACTACGCAAGTTTAGA
ATACTCACAACTGATCTTTCAAGTCTCCCCTGTGGATGTCCAAATTGTAT
AACAACTGCATCCAAAGAATTGCAAGGTTATCAAAGAAAGATGAAGGTC
CATATATGAATACAGTACACACAAACTATGTAAACACTACCATCAATGGA
CCAGAAGAGGGCAATGTCAATACAGAAGTACAAATGATGACAATGGAAG
CGATAAGCCTTATGATGATCTTGTAGAAGAAGCTTCTCGAAGTCTCGGAAA
AACCATATA
```

# Output del sequenziamento

```
>Mg_MxT01_06C10
GACACAAGTT AACAAAAT GACAGT GGT GT GT ACGGGT GCGACGACAGCGT
CAATTGCTT GGATT CCAGAT AAGACAGT AGTT CCT GGT GAAAATTTT ACA
GTGAAAT ACAAACAAACAGCGGACAAAT ACAACT ATT CCCTTTT AAAGA
ACCGT CAACT AAGGAT GACAT AT ATTT GCT ACAAGT AGACAGT CT CGCCC
CATCT ACAAAGT AT ACATTT ACATT GGAAT CCAAAAAT GGTTT AGGACGA
GCTGAAT CGAAT GAGT ACACAT GCACAACT AT GGCT GCAT CAGAGGAGT C
TGGAGTT AGT GGT GCT CT AAT AGGAGGAGTT GT ATT GAT AT CAATT GCGC
TACTGCT AAT CAT AAT AGT CATT GCGTT GATTTT ACT ACGCAAGTTT AGA
AT ACT CACAAC TAT CTTT CAAGT CT CCCCT GT GGAT GT CCAAATT GT AT
AACACGT GCAT CCAAAGAATT GCAAGTT AT CAAAGAAAGAT GAAGGT C
CAT AT AT GAAT ACAGT ACACACAACT AT GT AAACACT ACCAT CAAT GGA
CCAGAAGAGGGCAAT GT CAAT ACAGAAGT CACAAAT GAT GACAAT GGAAG
CGAT AAGCCTT AT GAT GAT CTT GT AAGAAGAACTT CGAACT CCT CGGAAA
AACCAT A
```



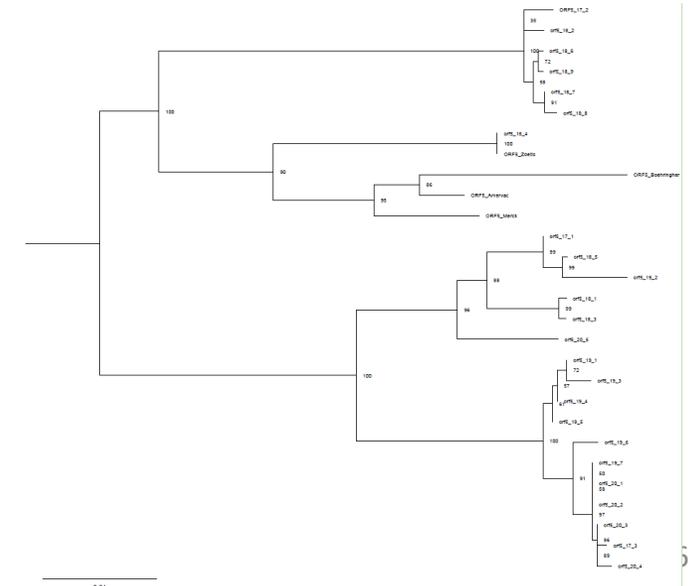
Elaborazione dei dati

Albero filogenetico

## PIM – tabelle di confronto

ORF5  
Percent Identity Matrix - created by Clustal2.1

			1	2	3	4	5	6	7	8
	1		100.00	83.28	83.88	84.19	89.11	94.07	99.67	88.45
	2		83.28	100.00	82.48	83.36	83.31	84.32	85.29	84.16
	3		83.88	82.48	100.00	99.17	84.77	84.96	84.77	85.62
	4		84.19	83.36	99.17	100.00	84.50	85.17	84.67	85.69
Vaccinale	5	ORF5_Boehringer	89.11	83.31	84.77	84.50	100.00	92.23	89.59	86.61
Vaccinale	6	ORF5_Amervac	94.07	84.32	84.96	85.17	92.23	100.00	94.21	88.60
Vaccinale	7	ORF5_Merck	99.67	85.29	84.77	84.67	89.59	94.21	100.00	88.93
Vaccinale	8	ORF5_Zoetis	88.45	84.16	85.62	85.69	86.61	88.60	88.93	100.00



## ● Analisi di sequenza

Lo scopo del confronto di due o più sequenze è quello di determinare se queste sono strettamente correlate o indipendenti



In altre parole: abbiamo a che fare con un virus «nuovo» o quello che circola è lo stipite «aziendale»???

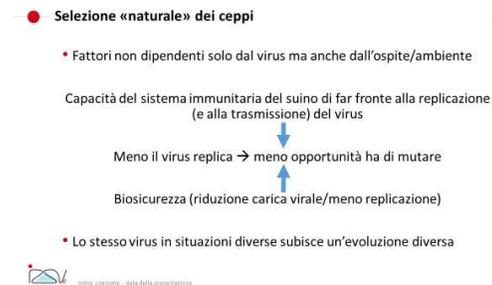
## ● Analisi di sequenza

Dobbiamo tenere a mente che la nostra analisi si basa solo su due geni:

- ORF 5 circa 600 nucleotidi, frequenza di mutazione/anno 0,5-1%
- ORF 7 circa 400 nucleotidi, più stabile rispetto a ORF 5

E' generalmente riconosciuto che confrontando 2 sequenze:

- strettamente correlate se percentuali di identità > 97-98% (CUT OFF)
- < 97-98%: indipendenti





## Attenzione

- Le differenze tra le varianti di un singolo ceppo circolante possono determinare il superamento del cut-off di differenza del 3%
- Il concetto di CUT-OFF è valido per il confronto di ceppi identificati in un determinato contesto ma non può essere applicato *tout-court* nello studio dell'evoluzione nel tempo dei ceppi presenti in un'azienda
- La disponibilità di informazioni anamnestiche quali data/sito di isolamento/gravità della sintomatologia rilevata sono di fondamentale importanza per capire se si tratta di un ceppo nuovo

# ● Output del sequenziamento – tabelle di confronto

ORF5							
Percent Identity Matrix - created by Clustal2.1							
			1	2	3	4	5
	1	orf5_	100.00				
Vaccinale	2	Orf5_Boehringher	82.51	100.00			
Vaccinale	3	Orf5_Amervac	83.20	92.41	100.00		
Vaccinale	4	Orf5_Merck	82.18	89.60	94.22	100.00	
Vaccinale	5	Orf5_Zoetis	83.17	86.63	88.94	89.11	100.00

ORF5										
Percent Identity Matrix - created by Clustal2.1										
			1	2	3	4	5	6	7	8
	1		100.00	83.28	83.88	84.19	89.11	94.07	99.67	88.45
	2		83.28	100.00	82.48	83.36	83.31	84.32	85.29	84.16
	3		83.88	82.48	100.00	99.17	84.77	84.96	84.77	85.62
	4		84.19	83.36	99.17	100.00	84.50	85.17	84.67	85.69
Vaccinale	5	ORF5_Boehringher	89.11	83.31	84.77	84.50	100.00	92.23	89.59	86.61
Vaccinale	6	ORF5_Amervac	94.07	84.32	84.96	85.17	92.23	100.00	94.21	88.60
Vaccinale	7	ORF5_Merck	99.67	85.29	84.77	84.67	89.59	94.21	100.00	88.93
Vaccinale	8	ORF5_Zoetis	88.45	84.16	85.62	85.69	86.61	88.60	88.93	100.00

# Output del sequenziamento – tabelle di confronto

Percent Identity Matrix - created by Clustal2.1

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1		100.00	87.17	94.17	83.83	93.33	84.17	93.33	83.00	93.33	83.83	83.00	83.36	83.81	89.32	92.13	89.33	88.83	89.17	87.67	87.83	87.83	87.67	87.67	87.50	99.50	83.17	83.50	83.33	83.00
2		87.17	100.00	88.61	81.02	88.85	81.19	88.50	82.51	87.79	80.86	80.17	80.31	80.97	96.99	87.27	96.37	97.03	97.17	97.69	99.17	99.17	99.01	99.34	99.00	87.35	82.01	83.17	82.01	82.51
3		94.17	88.61	100.00	83.17	95.83	82.54	95.83	83.63	99.27	82.92	82.81	83.36	83.31	89.25	97.23	88.97	88.66	89.70	87.87	88.46	88.44	88.26	88.17	88.85	94.34	83.50	84.68	83.00	83.33
4		83.83	81.02	83.17	100.00	82.36	98.02	82.33	83.99	82.67	98.35	97.69	97.62	98.33	81.47	82.58	81.52	81.68	81.70	81.19	81.52	81.52	81.68	81.35	81.03	83.86	84.32	84.16	83.66	83.99
5		93.33	88.85	95.83	82.36	100.00	81.28	99.33	82.29	95.40	81.55	81.36	81.83	82.47	89.74	93.47	89.62	89.10	90.20	88.58	88.67	88.62	88.42	88.30	89.18	93.51	82.20	83.22	81.86	82.20
6		84.17	81.19	82.54	98.02	81.28	100.00	82.50	83.36	82.01	98.75	98.02	97.96	98.66	81.60	82.71	81.67	81.48	82.23	80.97	81.51	81.72	81.49	81.27	81.20	84.53	84.82	85.01	84.49	84.32
7		93.33	88.50	95.83	82.33	99.33	82.50	100.00	82.33	95.33	82.17	81.33	81.83	82.47	89.98	93.63	90.00	89.67	89.83	89.33	89.17	89.17	89.00	89.00	88.83	93.50	82.33	83.33	82.17	82.33
8		83.00	82.51	83.63	83.99	82.29	83.36	82.33	100.00	83.38	83.70	84.30	84.72	84.47	82.08	82.06	82.33	82.38	82.56	82.12	82.74	82.79	82.51	82.58	82.03	83.03	86.63	88.96	89.11	100.00
9		93.33	87.79	99.27	82.67	95.40	82.01	95.33	83.38	100.00	82.45	82.31	82.85	82.80	88.44	97.72	88.52	87.92	88.87	87.04	87.72	87.70	87.52	87.33	88.02	93.51	83.00	84.18	82.51	83.17
10		83.83	80.86	82.92	98.35	81.55	98.75	82.17	83.70	82.45	100.00	98.68	98.64	99.67	81.27	82.38	81.28	81.34	81.89	80.91	81.22	81.28	81.00	81.06	80.87	84.19	84.65	85.17	84.65	84.65
11		83.00	80.17	82.81	97.69	81.36	98.02	81.33	84.30	82.31	98.68	100.00	98.81	98.66	80.97	81.91	80.99	80.99	81.20	80.33	80.66	80.66	80.50	80.50	80.20	83.36	84.30	84.79	84.46	84.30
12		83.36	80.31	83.36	97.62	81.83	97.96	81.83	84.72	82.85	98.64	98.81	100.00	98.64	81.32	82.51	81.15	80.81	81.15	80.14	80.81	80.81	80.65	80.65	80.48	83.53	84.55	85.06	84.55	84.72
13		83.81	80.97	83.31	98.33	82.47	98.66	82.47	84.47	82.80	99.67	98.66	98.64	100.00	81.64	82.75	81.47	81.64	81.80	81.14	81.47	81.47	81.30	81.30	81.14	84.14	84.64	84.97	84.47	84.47
14		89.32	96.99	89.25	81.47	89.74	81.60	89.98	82.08	88.44	81.27	80.97	81.32	81.64	100.00	88.07	99.19	99.51	99.50	96.91	97.56	97.56	97.39	97.39	97.16	89.48	82.80	83.50	82.64	82.64
15		92.13	87.27	97.23	82.58	93.47	82.71	93.63	82.06	97.72	82.38	81.91	82.51	82.75	88.07	100.00	88.09	87.60	88.46	86.62	87.44	87.44	87.44	87.28	87.60	92.29	83.25	84.11	82.41	82.24
16		89.33	96.37	88.97	81.52	89.62	81.67	90.00	82.33	88.52	81.28	80.99	81.15	81.47	99.19	88.09	100.00	98.79	98.67	96.21	96.97	96.98	96.68	96.67	96.34	89.52	82.84	83.36	82.51	82.34
17		88.83	97.03	88.66	81.68	89.10	81.48	89.67	82.38	87.92	81.34	80.99	80.81	81.64	99.51	87.60	98.79	100.00	99.67	97.19	97.78	97.78	97.47	97.48	97.17	89.02	82.67	83.20	82.34	82.34
18		89.17	97.17	89.70	81.70	90.20	82.23	89.83	82.56	88.87	81.89	81.20	81.15	81.80	99.50	88.46	98.67	99.67	100.00	97.84	98.01	98.01	97.84	97.84	97.34	89.35	83.03	83.39	82.53	82.53
19		87.67	97.69	87.87	81.19	88.58	80.97	89.33	82.12	87.04	80.91	80.33	80.14	81.14	96.91	86.62	96.21	97.19	97.84	100.00	98.22	98.22	97.91	97.94	97.84	87.85	82.01	83.20	82.01	82.18
20		87.83	99.17	88.46	81.52	88.67	81.51	89.17	82.74	87.72	81.22	80.66	80.81	81.47	97.56	87.44	96.97	97.78	98.01	98.22	100.00	100.00	99.70	99.70	99.33	88.02	82.01	83.20	82.01	82.67
21		87.83	99.17	88.44	81.52	88.62	81.72	89.17	82.79	87.70	81.28	80.66	80.81	81.47	97.56	87.44	96.98	97.78	98.01	98.22	100.00	100.00	99.70	99.70	99.33	88.02	82.01	83.20	82.01	82.67
22		87.67	99.01	88.26	81.68	88.42	81.49	89.00	82.51	87.52	81.00	80.50	80.65	81.30	97.39	87.44	96.68	97.47	97.84	97.91	99.70	99.70	100.00	99.40	99.17	87.85	81.85	83.03	81.85	82.51
23		87.67	99.34	88.17	81.35	88.30	81.27	89.00	82.58	87.33	81.06	80.50	80.65	81.30	97.39	87.28	96.67	97.48	97.84	97.94	99.70	99.70	99.40	100.00	99.50	87.85	82.18	83.36	82.18	82.51
24		87.50	99.00	88.85	81.03	89.18	81.20	88.83	82.03	88.02	80.87	80.20	80.48	81.14	97.16	87.60	96.34	97.17	97.34	97.84	99.33	99.33	99.17	99.50	100.00	87.69	82.03	82.70	81.86	82.03
25		99.50	87.35	94.34	83.86	93.51	84.53	93.50	83.03	93.51	84.19	83.36	83.53	84.14	89.48	92.29	89.52	89.02	89.35	87.85	88.02	88.02	87.85	87.85	87.69	100.00	83.36	83.69	83.36	83.03
26	ORF5_Boehringer	83.17	82.01	83.50	84.32	82.20	84.82	82.33	86.63	83.00	84.65	84.30	84.55	84.64	82.80	83.25	82.84	82.67	83.03	82.01	82.01	82.01	81.85	82.18	82.03	83.36	100.00	92.41	89.60	86.63
27	ORF5_Amervac	83.50	83.17	84.68	84.16	83.22	85.01	83.33	88.96	84.18	85.17	84.79	85.06	84.97	83.50	84.11	83.36	83.20	83.39	83.20	83.20	83.20	83.03	83.36	82.70	83.69	92.41	100.00	94.22	88.94
28	ORF5_Merck	83.33	82.01	83.00	83.66	81.86	84.49	82.17	89.11	82.51	84.65	84.46	84.55	84.47	82.64	82.41	82.51	82.34	82.53	82.01	82.01	82.01	81.85	82.18	81.86	83.36	89.60	94.22	100.00	89.11
29	ORF5_Zoetis	83.00	82.51	83.33	83.99	82.20	84.32	82.33	100.00	83.17	84.65	84.30	84.72	84.47	82.64	82.24	82.34	82.34	82.53	82.18	82.67	82.67	82.51	82.51	82.03	83.03	86.63	88.94	89.11	100.00

# Output del sequenziamento – tabelle di confronto

Percent Identity Matrix - created by Clustal2.1															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	100.00	87.17	94.17	83.83	93.33	84.17	93.33	83.00	93.33	83.83	83.00	83.36	83.81	89.32	92.1
2	87.17	100.00	88.61	81.02	88.85	81.19	88.50	82.51	87.79	80.86	80.17	80.31	80.97	96.99	87.2
3	94.17	88.61	100.00	83.17	95.83	82.54	95.83	83.63	99.27	82.92	82.81	83.36	83.31	89.25	97.2
4	83.83	81.02	83.17	100.00	82.36	98.02	82.33	83.99	82.67	98.35	97.69	97.62	98.33	81.47	82.5
5	93.33	88.85	95.83	82.36	100.00	81.28	99.33	82.29	95.40	81.55	81.36	81.83	82.47	89.74	93.4
6	84.17	81.19	82.54	98.02	81.28	100.00	82.50	83.36	82.01	98.75	98.02	97.96	98.66	81.60	82.7
7	83.33	88.50	95.83	82.33	88.85	82.50	100.00	83.33	85.27	83.17	81.33	81.83	82.47	89.74	93.6
8	83.83	81.19	82.54	98.02	81.28	100.00	82.50	83.36	82.01	98.75	98.02	97.96	98.66	81.60	82.7
9	93.33	88.85	95.83	82.36	100.00	81.28	99.33	82.29	95.40	81.55	81.36	81.83	82.47	89.74	93.4
10	84.17	81.19	82.54	98.02	81.28	100.00	82.50	83.36	82.01	98.75	98.02	97.96	98.66	81.60	82.7
11	83.33	88.50	95.83	82.33	88.85	82.50	100.00	83.33	85.27	83.17	81.33	81.83	82.47	89.74	93.6
12	83.83	81.19	82.54	98.02	81.28	100.00	82.50	83.36	82.01	98.75	98.02	97.96	98.66	81.60	82.7
13	93.33	88.85	95.83	82.36	100.00	81.28	99.33	82.29	95.40	81.55	81.36	81.83	82.47	89.74	93.4
14	84.17	81.19	82.54	98.02	81.28	100.00	82.50	83.36	82.01	98.75	98.02	97.96	98.66	81.60	82.7
15	83.33	88.50	95.83	82.33	88.85	82.50	100.00	83.33	85.27	83.17	81.33	81.83	82.47	89.74	93.6

ORF5 Percent Identity Matrix - created by Clustal2.1								
	1	2	3	4	5	6	7	8
1	100.00	83.28	83.88	84.19	89.11	94.07	99.67	88.45
2	83.28	100.00	82.48	83.36	83.31	84.32	85.29	84.16
3	83.88	82.48	100.00	99.17	84.77	84.96	84.77	85.62
4	84.19	83.36	99.17	100.00	84.50	85.17	84.67	85.69
5	89.11	83.31	84.77	84.50	100.00	92.23	89.59	86.61
6	94.07	84.32	84.96	85.17	92.23	100.00	94.21	88.60
7	99.67	85.29	84.77	84.67	89.59	94.21	100.00	88.93
8	88.45	84.16	85.62	85.69	86.61	88.60	88.93	100.00

ORF5 Percent Identity Matrix - created by Clustal2.1					
	1	2	3	4	5
1	100.00				
2	82.51	100.00			
3	83.20	92.41	100.00		
4	82.18	89.60	94.22	100.00	
5	83.17	86.63	88.94	89.11	100.00

		1	2	3	4	5
Vaccinale	1	100.00				
Vaccinale	2	82.51	100.00			
Vaccinale	3	83.20	92.41	100.00		
Vaccinale	4	82.18	89.60	94.22	100.00	
Vaccinale	5	83.17	86.63	88.94	89.11	100.00

Percent Identity Matrix - created by Clustal2.1																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15															
22	87.67	99.01	88.26	81.68	88.42	81.49	89.00	82.51	87.52	81.00	80.50	80.65	81.30	97.39	87.44	96.68	97.47	97.84	97.91	99.70	99.70	100.00	99.40	99.17	87.85	81.85	83.03	81.85	82.51	
23	87.67	99.34	88.17	81.35	88.30	81.27	89.00	82.58	87.33	81.06	80.50	80.65	81.30	97.39	87.28	96.67	97.48	97.84	97.94	99.70	99.70	99.40	100.00	99.50	87.85	82.18	83.36	82.18	82.51	
24	87.50	99.00	88.85	81.03	89.18	81.20	88.83	82.03	88.02	80.87	80.20	80.48	81.14	97.16	87.60	96.34	97.17	97.34	97.84	99.33	99.33	99.17	99.50	100.00	87.69	82.03	82.70	81.86	82.03	
25	99.50	87.35	94.34	83.86	93.51	84.53	93.50	83.03	93.51	84.19	83.36	83.53	84.14	89.48	92.29	89.52	89.02	89.35	87.85	88.02	88.02	87.85	87.85	87.69	100.00	83.36	83.69	83.36	83.03	
26	ORF5_Boehringer	83.17	82.01	83.50	84.32	82.20	84.82	82.33	86.63	83.00	84.65	84.30	84.55	84.64	82.80	83.25	82.84	82.67	83.03	82.01	82.01	82.01	81.85	82.18	82.03	83.36	100.00	92.41	89.60	86.63
27	ORF5_Amervac	83.50	83.17	84.68	84.16	83.22	85.01	83.33	88.96	84.18	85.17	84.79	85.06	84.97	83.50	84.11	83.36	83.20	83.39	83.20	83.20	83.20	83.03	83.36	82.70	83.69	92.41	100.00	94.22	88.94
28	ORF5_Merck	83.33	82.01	83.00	83.66	81.86	84.49	82.17	89.11	82.51	84.65	84.46	84.55	84.47	82.64	82.41	82.51	82.34	82.53	82.01	82.01	82.01	81.85	82.18	81.86	83.36	89.60	94.22	100.00	89.11
29	ORF5_Zoetis	83.00	82.51	83.33	83.99	82.20	84.32	82.33	100.00	83.17	84.65	84.30	84.72	84.47	82.64	82.24	82.34	82.34	82.53	82.18	82.67	82.67	82.51	82.51	82.03	83.03	86.63	88.94	89.11	100.00

**Pregi**

**Difetti**

Elaborazione rapida

Non gestibile con numeri elevati di sequenze

Immediatezza di lettura

Nessuna informazione evolutiva

Confronto tra poche sequenze

Dubbi interpretativi per valori prossimi al cut-off



# Output del sequenziamento – Albero filogenetico

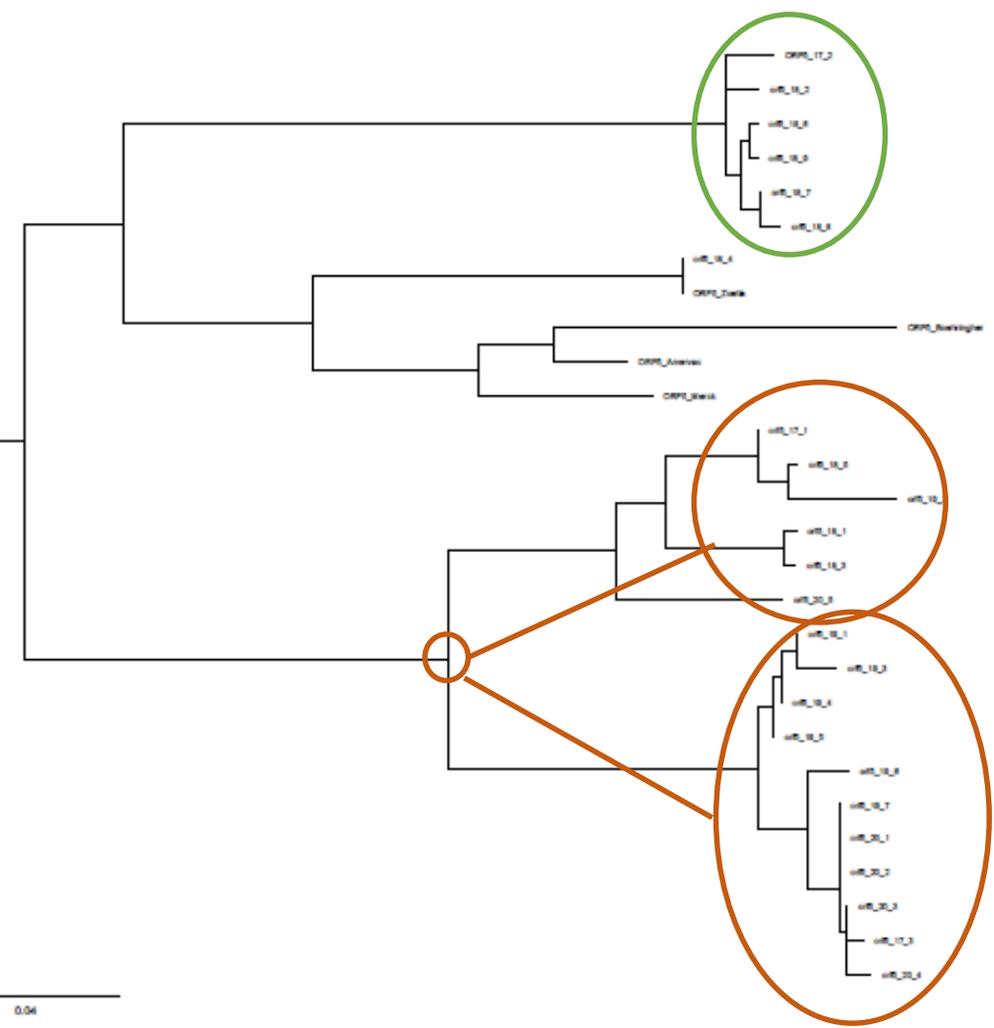
Percent Identity Matrix - created by Clustal2.1

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1		100.00	87.17	94.17	83.83	93.33	84.17	93.33	83.00	93.33	83.83	83.00	83.36	83.81	89.32	92.13	89.33	88.83	89.17	87.67	87.83	87.83	87.67	87.67	87.50	99.50	83.17	83.50	83.33	83.00
2		87.17	100.00	88.61	81.02	88.85	81.19	88.50	82.51	87.79	80.86	80.17	80.31	80.97	96.99	87.27	96.37	97.03	97.17	97.69	99.17	99.17	99.01	99.34	99.00	87.35	82.01	83.17	82.01	82.51
3		94.17	88.61	100.00	83.17	95.83	82.54	95.83	83.63	99.27	82.92	82.81	83.36	83.31	89.25	97.23	88.97	88.66	89.70	87.87	88.46	88.44	88.26	88.17	88.85	94.34	83.50	84.68	83.00	83.33
4		83.83	81.02	83.17	100.00	82.36	98.02	82.33	83.99	82.67	98.35	97.69	97.62	98.33	81.47	82.58	81.52	81.68	81.70	81.19	81.52	81.52	81.68	81.35	81.03	83.86	84.32	84.16	83.66	83.99
5		93.33	88.85	95.83	82.36	100.00	81.28	99.33	82.29	95.40	81.55	81.36	81.83	82.47	89.74	93.47	89.62	89.10	90.20	88.58	88.67	88.62	88.42	88.30	89.18	93.51	82.20	83.22	81.86	82.20
6		84.17	81.19	82.54	98.02	81.28	100.00	82.50	83.36	82.01	98.75	98.02	97.96	98.66	81.60	82.71	81.67	81.48	82.23	80.97	81.51	81.72	81.49	81.27	81.20	84.53	84.82	85.01	84.49	84.32
7		93.33	88.50	95.83	82.33	99.33	82.50	100.00	82.33	95.33	82.17	81.33	81.83	82.47	89.98	93.63	90.00	89.67	89.83	89.33	89.17	89.17	89.00	89.00	88.83	93.50	82.33	83.33	82.17	82.33
8		83.00	82.51	83.63	83.99	82.29	83.36	82.33	100.00	83.38	83.70	84.30	84.72	84.47	82.08	82.06	82.33	82.38	82.56	82.12	82.74	82.79	82.51	82.58	82.03	83.03	86.63	88.96	89.11	100.00
9		93.33	87.79	99.27	82.67	95.40	82.01	95.33	83.38	100.00	82.45	82.31	82.85	82.80	88.44	97.72	88.52	87.92	88.87	87.04	87.72	87.70	87.52	87.33	88.02	93.51	83.00	84.18	82.51	83.17
10		83.83	80.86	82.92	98.35	81.55	98.75	82.17	83.70	82.45	100.00	98.68	98.64	99.67	81.27	82.38	81.28	81.34	81.89	80.91	81.22	81.28	81.00	81.06	80.87	84.19	84.65	85.17	84.65	84.65
11		83.00	80.17	82.81	97.69	81.36	98.02	81.33	84.30	82.31	98.68	100.00	98.81	98.66	80.97	81.91	80.99	80.99	81.20	80.33	80.66	80.66	80.50	80.50	80.20	83.36	84.30	84.79	84.46	84.30
12		83.36	80.31	83.36	97.62	81.83	97.96	81.83	84.72	82.85	98.64	98.81	100.00	98.64	81.32	82.51	81.15	80.81	81.15	80.14	80.81	80.81	80.65	80.65	80.48	83.53	84.55	85.06	84.55	84.72
13		83.81	80.97	83.31	98.33	82.47	98.66	82.47	84.47	82.80	99.67	98.66	98.64	100.00	81.64	82.75	81.47	81.64	81.80	81.14	81.47	81.47	81.30	81.30	81.14	84.14	84.64	84.97	84.47	84.47
14		89.32	96.99	89.25	81.47	89.74	81.60	89.98	82.08	88.44	81.27	80.97	81.32	81.64	100.00	88.07	99.19	99.51	99.50	96.91	97.56	97.56	97.39	97.39	97.16	89.48	82.80	83.50	82.64	82.64
15		92.13	87.27	97.23	82.58	93.47	82.71	93.63	82.06	97.72	82.38	81.91	82.51	82.75	88.07	100.00	88.09	87.60	88.46	86.62	87.44	87.44	87.44	87.28	87.60	92.29	83.25	84.11	82.41	82.24
16		89.33	96.37	88.97	81.52	89.62	81.67	90.00	82.33	88.52	81.28	80.99	81.15	81.47	99.19	88.09	100.00	98.79	98.67	96.21	96.97	96.98	96.68	96.67	96.34	89.52	82.84	83.36	82.51	82.34
17		88.83	97.03	88.66	81.68	89.10	81.48	89.67	82.38	87.92	81.34	80.99	80.81	81.64	99.51	87.60	98.79	100.00	99.67	97.19	97.78	97.78	97.47	97.48	97.17	89.02	82.67	83.20	82.34	82.34
18		89.17	97.17	89.70	81.70	90.20	82.23	89.83	82.56	88.87	81.89	81.20	81.15	81.80	99.50	88.46	98.67	99.67	100.00	97.84	98.01	98.01	97.84	97.84	97.34	89.35	83.03	83.39	82.53	82.53
19		87.67	97.69	87.87	81.19	88.58	80.97	89.33	82.12	87.04	80.91	80.33	80.14	81.14	96.91	86.62	96.21	97.19	97.84	100.00	98.22	98.22	97.91	97.94	97.84	87.85	82.01	83.20	82.01	82.18
20		87.83	99.17	88.46	81.52	88.67	81.51	89.17	82.74	87.72	81.22	80.66	80.81	81.47	97.56	87.44	96.97	97.78	98.01	98.22	100.00	100.00	99.70	99.70	99.33	88.02	82.01	83.20	82.01	82.67
21		87.83	99.17	88.44	81.52	88.62	81.72	89.17	82.79	87.70	81.28	80.66	80.81	81.47	97.56	87.44	96.98	97.78	98.01	98.22	100.00	100.00	99.70	99.70	99.33	88.02	82.01	83.20	82.01	82.67
22		87.67	99.01	88.26	81.68	88.42	81.49	89.00	82.51	87.52	81.00	80.50	80.65	81.30	97.39	87.44	96.68	97.47	97.84	97.91	99.70	99.70	100.00	99.40	99.17	87.85	81.85	83.03	81.85	82.51
23		87.67	99.34	88.17	81.35	88.30	81.27	89.00	82.58	87.33	81.06	80.50	80.65	81.30	97.39	87.28	96.67	97.48	97.84	97.94	99.70	99.70	99.40	100.00	99.50	87.85	82.18	83.36	82.18	82.51
24		87.50	99.00	88.85	81.03	89.18	81.20	88.83	82.03	88.02	80.87	80.20	80.48	81.14	97.16	87.60	96.34	97.17	97.34	97.84	99.33	99.33	99.17	99.50	100.00	87.69	82.03	82.70	81.86	82.03
25		99.50	87.35	94.34	83.86	93.51	84.53	93.50	83.03	93.51	84.19	83.36	83.53	84.14	89.48	92.29	89.52	89.02	89.35	87.85	88.02	88.02	87.85	87.85	87.69	100.00	83.36	83.69	83.36	83.03
26	ORF5_Boehringer	83.17	82.01	83.50	84.32	82.20	84.82	82.33	86.63	83.00	84.65	84.30	84.55	84.64	82.80	83.25	82.84	82.67	83.03	82.01	82.01	82.01	81.85	82.18	82.03	83.36	100.00	92.41	89.60	86.63
27	ORF5_Amervac	83.50	83.17	84.68	84.16	83.22	85.01	83.33	88.96	84.18	85.17	84.79	85.06	84.97	83.50	84.11	83.36	83.20	83.39	83.20	83.20	83.20	83.03	83.36	82.70	83.69	92.41	100.00	94.22	88.94
28	ORF5_Merck	83.33	82.01	83.00	83.66	81.86	84.49	82.17	89.11	82.51	84.65	84.46	84.55	84.47	82.64	82.41	82.51	82.34	82.53	82.01	82.01	82.01	81.85	82.18	81.86	83.36	89.60	94.22	100.00	89.11
29	ORF5_Zoetis	83.00	82.51	83.33	83.99	82.20	84.32	82.33	100.00	83.17	84.65	84.30	84.72	84.47	82.64	82.24	82.34	82.34	82.53	82.18	82.67	82.67	82.51	82.51	82.03	83.03	86.63	88.94	89.11	100.00

# Output del sequenziamento – Albero filogenetico

Percent Identity Matrix - created by Clustal2.1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29				
1	100.00	87.17	94.17	83.83	93.33	84.17	93.33	83.00	91.33	83.83	80.00	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83		
2	87.17	100.00	88.61	81.02	88.83	81.59	88.50	82.56	82.56	87.79	80.86	80.17	80.11	80.57	80.99	87.27	86.37	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	87.03	
3	94.17	88.61	100.00	81.17	83.86	83.83	86.52	91.27	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
4	83.83	81.02	81.17	100.00	82.56	82.56	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	
5	93.33	88.83	93.33	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
6	84.17	81.59	83.86	94.17	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
7	93.33	88.50	93.33	83.83	93.33	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
8	83.00	82.56	83.83	83.83	82.56	82.56	83.83	100.00	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	83.83	
9	91.33	87.79	91.27	82.56	82.56	82.56	83.83	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
10	83.83	80.86	82.56	83.83	82.56	82.56	83.83	83.83	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
11	83.00	80.17	82.56	82.56	82.56	82.56	83.83	83.83	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
12	81.36	80.51	83.86	82.56	82.56	82.56	83.83	82.56	82.56	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
13	81.81	80.97	83.25	83.83	82.47	86.86	82.47	86.87	82.80	86.87	86.86	82.75	100.00	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	
14	91.27	86.99	89.25	82.47	87.26	82.80	82.80	82.80	82.80	82.80	82.80	82.75	82.75	100.00	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	82.75	
15	92.11	87.27	92.23	82.56	82.47	82.71	83.83	82.06	82.71	82.38	82.38	82.38	82.38	82.38	100.00	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	
16	89.33	86.37	86.97	81.52	82.82	82.82	82.82	82.82	82.82	82.82	82.82	82.82	82.82	82.82	82.38	100.00	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	
17	88.83	87.03	88.83	81.48	82.80	82.80	82.80	82.80	82.80	82.80	82.80	82.80	82.80	82.80	82.38	82.38	100.00	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	
18	89.17	87.17	89.70	83.76	80.20	82.23	83.83	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.38	100.00	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	82.38	
19	87.67	87.69	87.67	82.19	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
20	87.69	89.17	86.86	81.52	82.47	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
21	87.83	89.17	86.86	81.52	82.47	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
22	87.67	89.01	86.26	82.48	82.48	82.49	82.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
23	87.67	89.06	86.17	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	
24	87.50	89.00	86.00	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	82.56	100.00	82.56	82.56	82.56	82.56	82.56	82.56	
25	90.26	87.05	84.34	81.86	81.51	84.31	81.50	82.03	81.51	84.19	81.36	81.53	84.14	84.48	82.29	82.52	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	100.00	82.03	82.03	82.03	82.03	82.03	
26	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	82.03	100.00	82.03	82.03	82.03	82.03	
27	CHS_America	81.30	81.17	84.98	84.16	82.52	82.51	81.33	82.96	84.18	85.17	84.79	82.09	84.97	81.50	84.11	83.36	83.20	83.20	83.20	83.20	83.20	83.20	83.20	83.20	83.20	83.20	82.03	100.00	84.22	84.94	84.94	
28	CHS_Africa	81.01	81.01	81.86	82.86	82.86	82.86	81.11	82.11	82.11	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.05	84.22	100.00	84.11	84.11	84.11	
29	CHS_India	81.00	82.51	83.33	83.89	82.24	82.33	82.33	82.33	82.33	84.05	84.30	84.72	84.47	82.84	82.84	82.84	82.84	82.84	82.84	82.84	82.84	82.84	82.84	82.84	82.84	82.84	84.11	84.11	100.00	84.11	84.11	84.11



<b>Pregi</b>	<b>Difetti</b>
Informazioni evolutive	Elaborazione complessa
Permette di gestire numeri elevati di sequenze	Richiede numeri elevati di sequenze
ci sono tanto più informativo è l'albero)	Necessario expertise per l'interpretazione

## ● Va tenuto in considerazione che...

- PIM e alberi filogenetici si basano su **stime** e su **informazioni incomplete** in quanto:
  - ✓ si basano su 1/15 del genoma (solo ORF5 e ORF7)
  - ✓ Non è possibile conoscere tutti gli stipti coinvolti in un determinato passaggio evolutivo (PRRSV ha tasso di mutazione elevato e possiamo studiare dal punto di vista filogenetico solo quelli che vengono conferiti per le analisi e spesso solo una parte di questi)
- Negli studi filogenetici è importante includere un dataset di sequenze rappresentative delle realtà locali/regionali/nazionali... mondiali
- Cattiva qualità delle sequenze, o impropria selezione del set di referenza possono portare a conclusioni errate

# ● Applicazioni dell'analisi di sequenza

- Non è uno strumento diagnostico
- E' uno strumento epidemiologico, utile per il monitoraggio dei ceppi circolanti in azienda/sito produttivo, gruppo integrato/regione o area geografica
- Potenzialità elevate... in funzione di
  - livello di condivisione dei dati (quantità di sequenze, n. di siti da cui provengono, areale coperto)
  - qualità dei dati (sequenze e informazioni cliniche/anamnestiche)



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Veterinary Microbiology 114 (2006) 214–224

veterinary  
microbiology

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Phylogenetic analysis of ORF5 and ORF7 sequences of porcine reproductive and respiratory syndrome virus (PRRSV) from PRRS-positive Italian farms: A showcase for PRRSV epidemiology and its consequences on farm management<sup>S</sup>

Patrizia Pesente<sup>a\*</sup>, Valeria Rebonato<sup>b</sup>, Gianpietro Sandri<sup>c</sup>,  
Davide Giovanardi<sup>a</sup>, Luigi Sperati Ruffoni<sup>a</sup>, Sandra Torriani<sup>b</sup>

<sup>a</sup>Laboratorio Tre Valli, Corto Pellegrina, San Martino Buon Albergo, 37036 Verona, Italy

<sup>b</sup>Dipartimento Scientifico e Tecnologico, Università degli Studi di Verona, Strada La Grazie 15, 37134 Verona, Italy

<sup>c</sup>Montorsi Francesco & Figli S.p.A., Magreta di Formigine, 41010 Modena, Italy

Received 28 June 2005; received in revised form 10 November 2005; accepted 15 November 2005

## ● 2 miti da sfatare....

- Confronto con ceppi vaccinali
  - utile per differenziare il virus vaccinale da quello aziendale in soggetti viremici post-vaccinazione
  - Non fornisce nessuna indicazione sulla scelta/efficacia di un vaccino
- Sequenziamento non da informazioni su patogenicità dello stipite
  - eventuali marker di patogenicità non sono ancora stati individuati

Grazie per l'attenzione