Next Generation Sequencing – The Role of New Sequence Technologies in Shaping the Future of Veterinary Science

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African swine fever

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New CL4 Laboratory Complex being built at IAH Pirbright

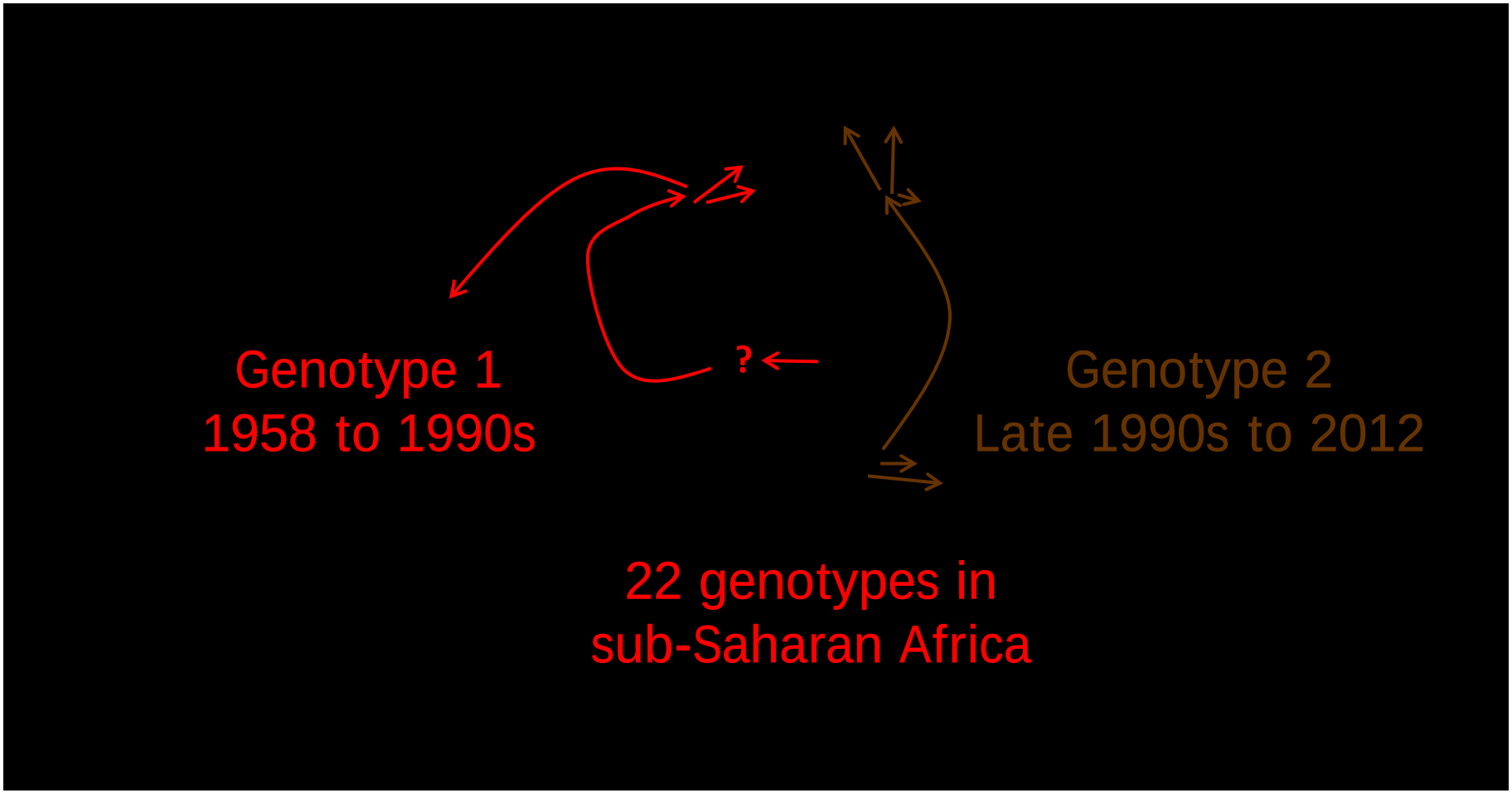
History of African swine fever: Africa

- Disease described in E. Africa as acute haemorrhagic fever with high mortality in domestic pigs (Montgomery 1921)
- Source of infection infected warthogs which had contact with domestic pigs but did not show clinical signs
- Further reports of ASF in pigs from E and S Africa where ASFV has been present in wildlife hosts for a very long time
- Subsequent spread through central and W Africa (reported 1950s) and to Indian Ocean islands of Madagascar (1998) and Mauritius (2007)

African swine fever

- Causes haemorrhagic fever with mortality up to ≈100% in pigs
- Natural hosts warthogs, bushpigs and soft ticks, persistently infected with no disease signs
- Endemic in many African countries and in Sardinia. Introduced to Georgia, Armenia, Azerbaijan, Russia 2007 spread to 16 states by 2011
- Major outbreaks kill high proportion of pigs, eg 30% pigs Ivory Coast (1996), 20% pigs Benin (1997), 50% pigs Madagascar (1998)
- No vaccine. Control relies on rapid diagnosis, implementation of quarantine and slaughter of pigs

Geographic spread of ASFV

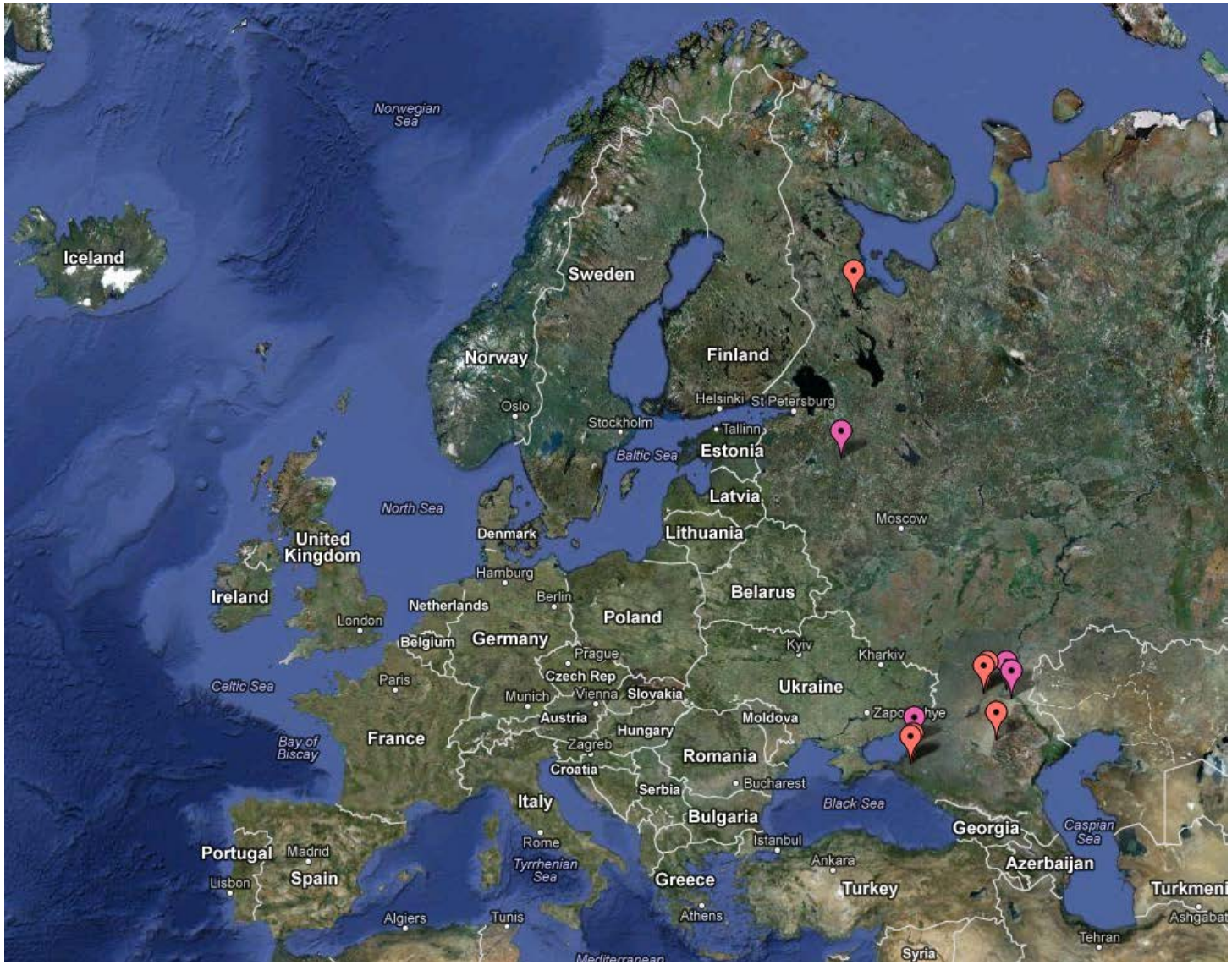


Outbreaks of ASFV in 2011

Pigs

Wild species

Outbreaks of ASFV in 2012

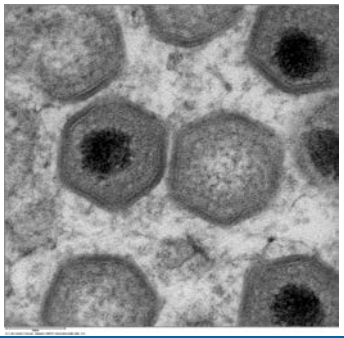


Pigs

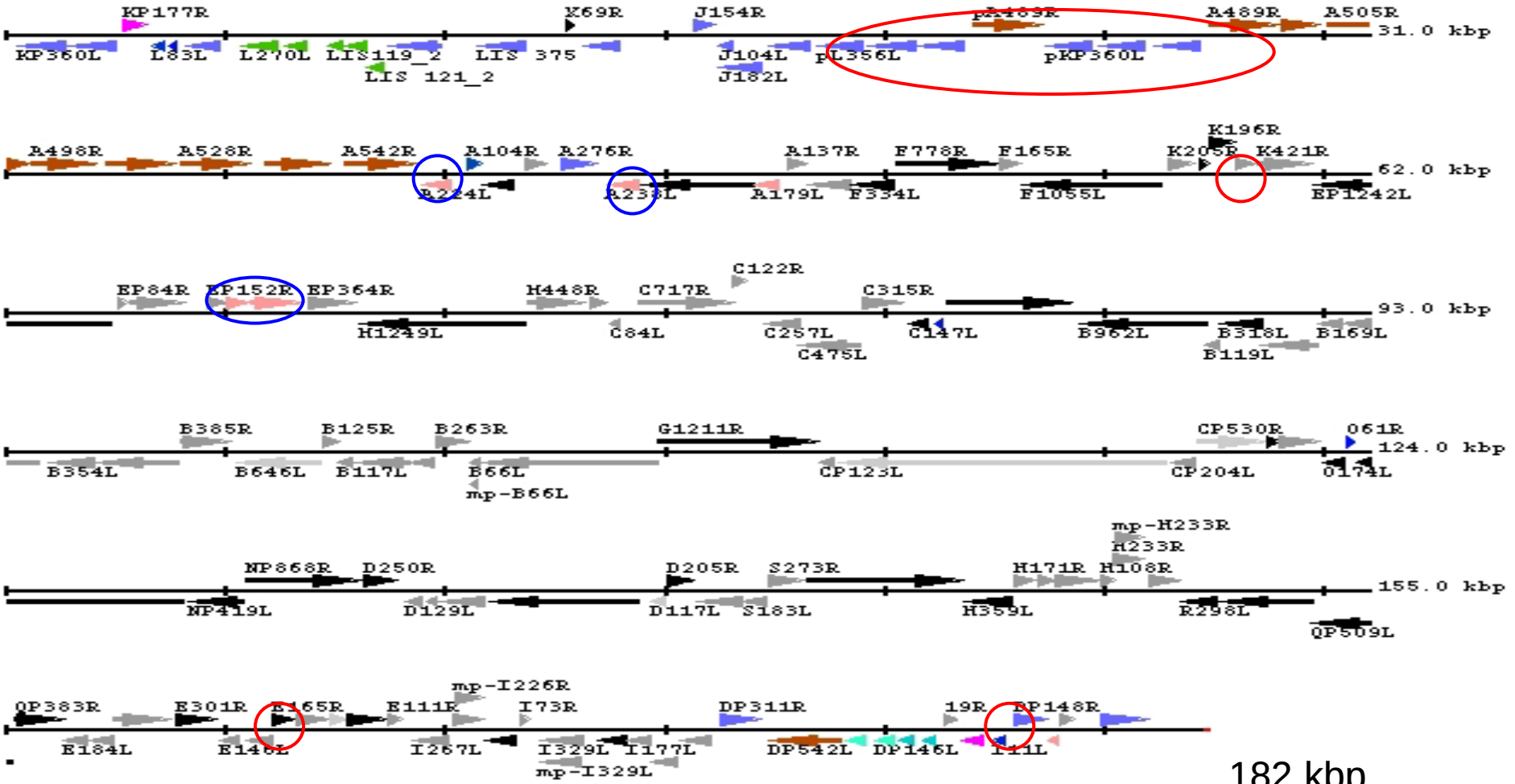
Wild species

African swine fever virus

- Large double-stranded DNA virus, genome length 170-190 kbp
- Only member of virus family the *Asfarviridae*
- Replicates in the cytoplasm – similar strategy to Poxviruses
- Virus particle contains RNA polymerase and other enzymes needed to start replication cycle – virus DNA is not infectious
- Encodes about 151-167 genes including enzymes required for replication and transcription of the virus genome
- Many genes are not essential for virus replication in cells but play an important role in virus survival and transmission
- Replicates mainly in macrophages *in vivo*



Function of genes encoded by ASFV



Deletion reduces virulence

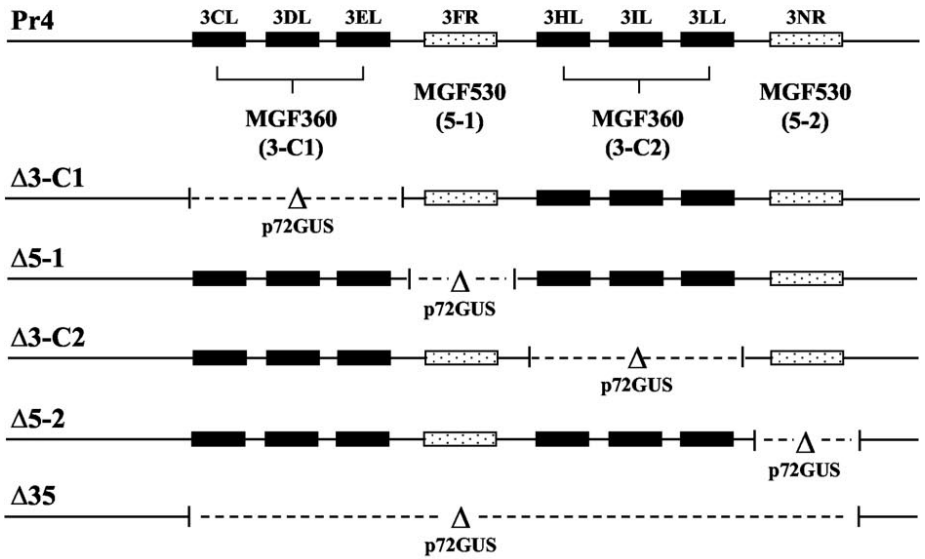
Deletion of evasion genes
-no reduction in virulence

Replication **MGF100** **MGF360**
Structural **P22** **MGF110**
unknown **evasion** **MGF505/530**

ASFV Multigene families

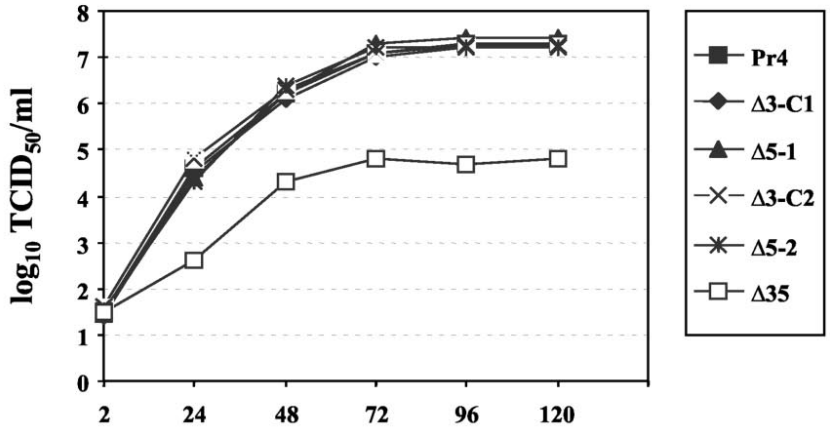
- 5 Multigene Families (MGFs)
  - A set of genes derived by duplication of an ancestral gene followed by independent mutational events resulting in a series of independent genes
- Constitute ~17% - 25% of the coding capacity
- Lack similarity to other known genes, functions unknown
- Vary in gene number between ASFV isolates:
  - MGF 100: 2-3 genes per genome
  - MGF 110: 5-13 genes per genome
  - MGF 300: 3-4 genes per genome
  - MGF 360: 11-19 genes per genome
  - MGF 530: 8-10 genes per genome

Deletion of MGF360 and MGF530 reduces virus growth in macrophages and virulence in pigs



	macrophage replication	virulence in pigs	tick replication	IFN induction
Pr4	+	+	+	-
Δ3-C1	+	NT	+	NT
Δ5-1	+	NT	+	NT
Δ3-C2	+	NT	↓ 10^2-10^3	NT
Δ5-2	+	NT	+	
Δ35	↓ 10^2-10^3	↓	↓ 10^2-10^3	↑

B



Note these MGF 360 and 530 genes are also deleted from non-pathogenic isolate (Chapman et al., 2008)

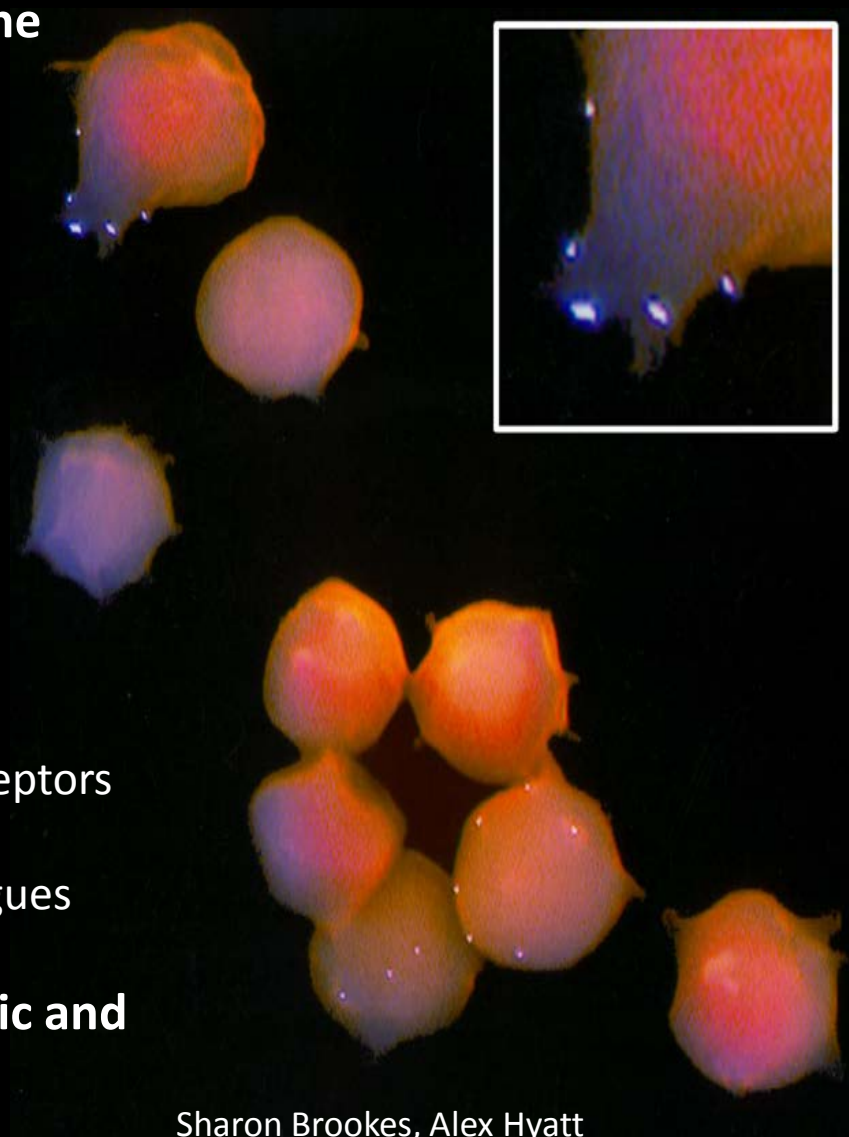
Zsak et al., 2001, Neilan et al., 2002, Afonso et al, Burrage et al.,2004

Identification of genes involved in immune evasion/virulence

Functional analysis of virus encoded immune evasion/virulence genes

- Inhibitors of host signalling pathways;
  A238L, inhibits host pathways (NF-κB calcineurin, p300) predicted broad inhibition of host gene transcription.
  -DP71L dephosphorylation of eIF-2$^{\alpha}$
  - Inhibitors of IFN
  -Inhibitor of Toll-like receptors TLR 3 and 4
- Adhesion proteins
  CD2v, causes binding of infected cells and virus particles to red blood cells, impairs lymphocyte proliferation
  C-type lectin -resembles NK cell inhibitory receptors inhibits MHC Class I surface expression
- Apoptosis inhibitors – IAP and Bcl2 homologues

Comparison of sequences of non-pathogenic and pathogenic strains



Sharon Brookes, Alex Hyatt

ASFV control measures

- Slaughter

- Experimental vaccine provides solid protection against homologous challenge, but genetic differences between pigs may play a role (MHC)
- Protection against heterologous challenge is more problematic
- DNA vaccination?

Sequencing of ASFV Georgia 2007/1 isolate

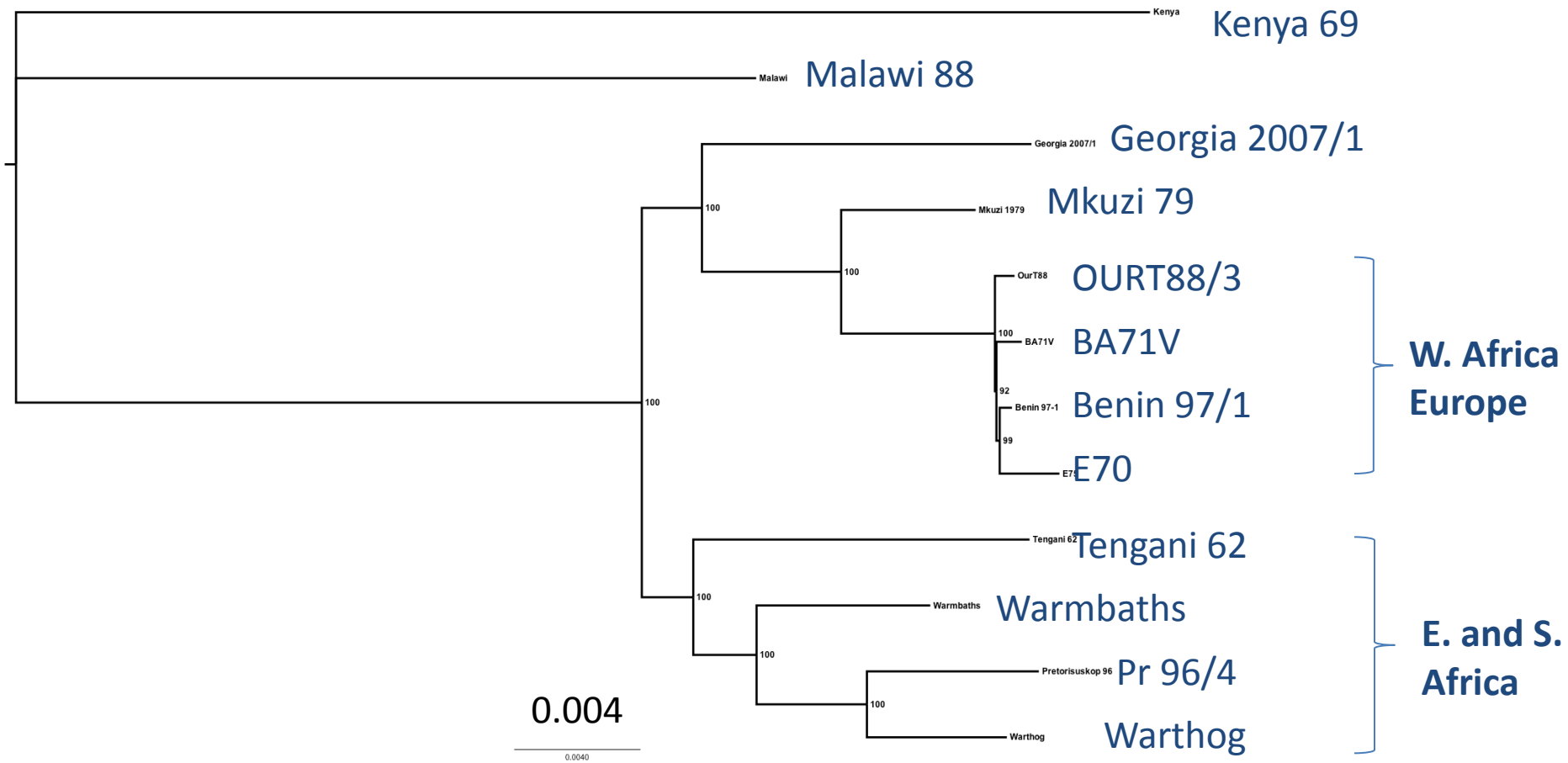
- To provide information for studies on gene function, information for vaccine development and to monitor genome evolution
- Virus from clinical samples grown in primary pig bone marrow cells and virus semi-purified from cell supernatants
- Contaminating cellular DNA removed by digestion with DNase then virus particles lysed with SDS and DNA extracted.
- Low molecular weight contaminants removed by filtration (Whatman Elu-Quick) and genome amplified by (Qiagen Repli-G kit).
- Genome sequenced and assembled at Liverpool (Roche 454 GSFLX) funded by RCVS Charitable Trust Grant

Chapman, Darby et al., 2011 Emerging Infectious Diseases

ASFV Genome Sequences

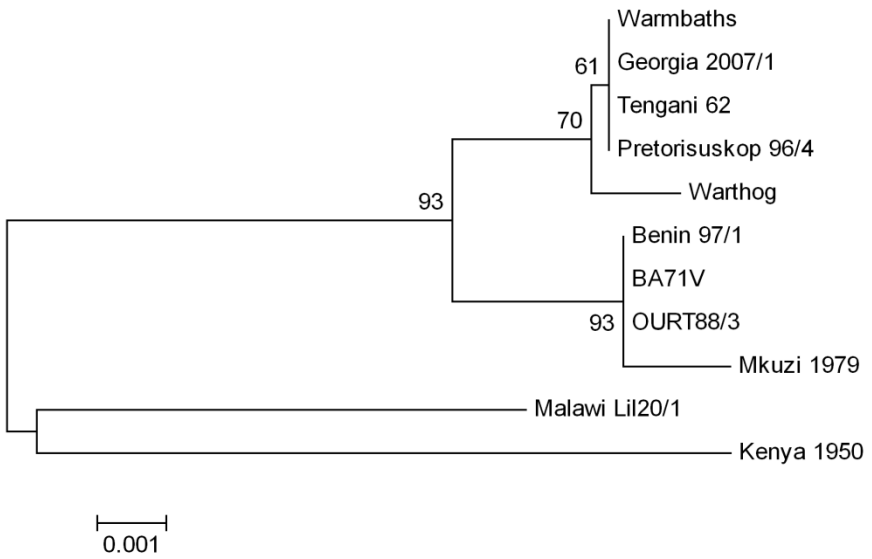
- ASFV Georgia 2007/1 sequence 189.3 kbp and encodes 166 open reading frames (ORFs)
- Sequences of virulent isolates vary from 182.3 to 193.9 kbp and encode 156-167 ORFs
- Sequences of non-virulent isolates is shorter – 170 to 171.7 kbp and encode 151-157 ORFs
- Most length variation is close to termini and results from gain or loss of genes from 5 different multigene families

Comparison of complete genomes of Georgia 2007/1 isolate with other ASFV isolates

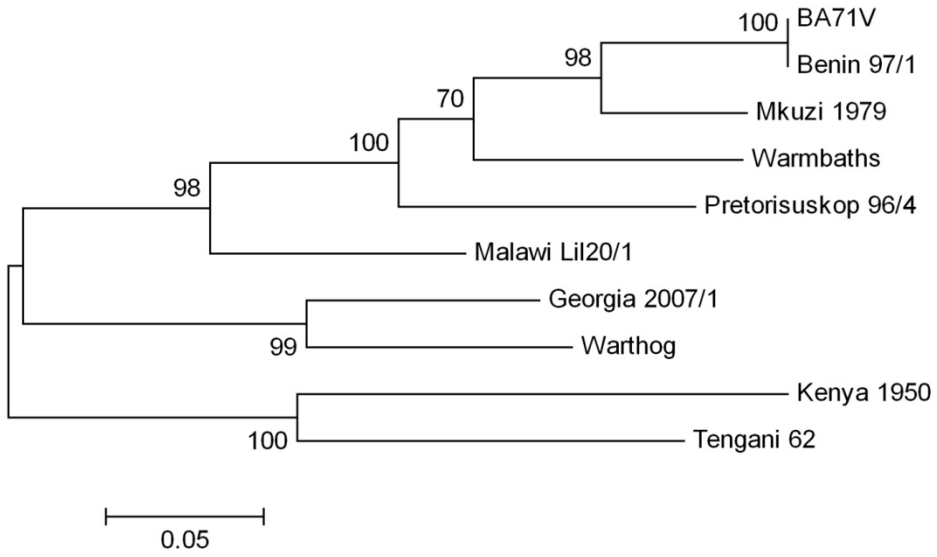


Comparison of the concatenated sequences of 125 conserved genes (~40,000 amino acids) shows the Georgia 2007 isolate is in the same clade as those from Europe and W. Africa but more distantly related -Chapman et al., Emerging Infectious Diseases 2011

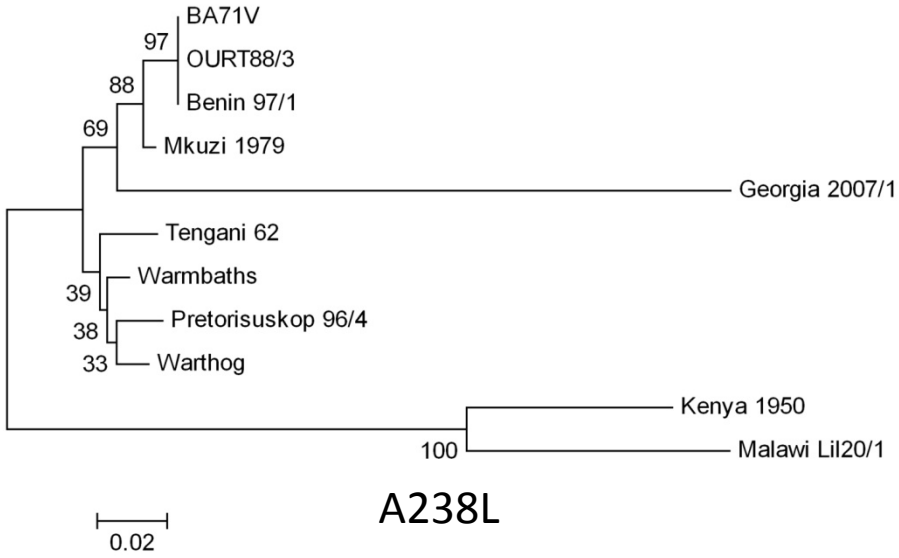
Phylogeny of individual ORFs can vary from that of 125 concatenated genes – evidence for recombination or selection pressure



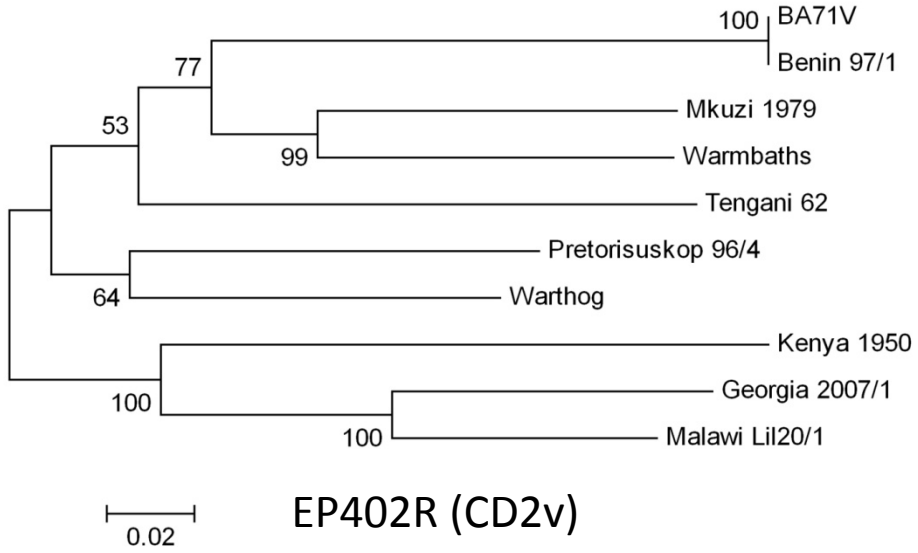
B646L (VP72, VP73)



EP153R (C-Type Lectin)

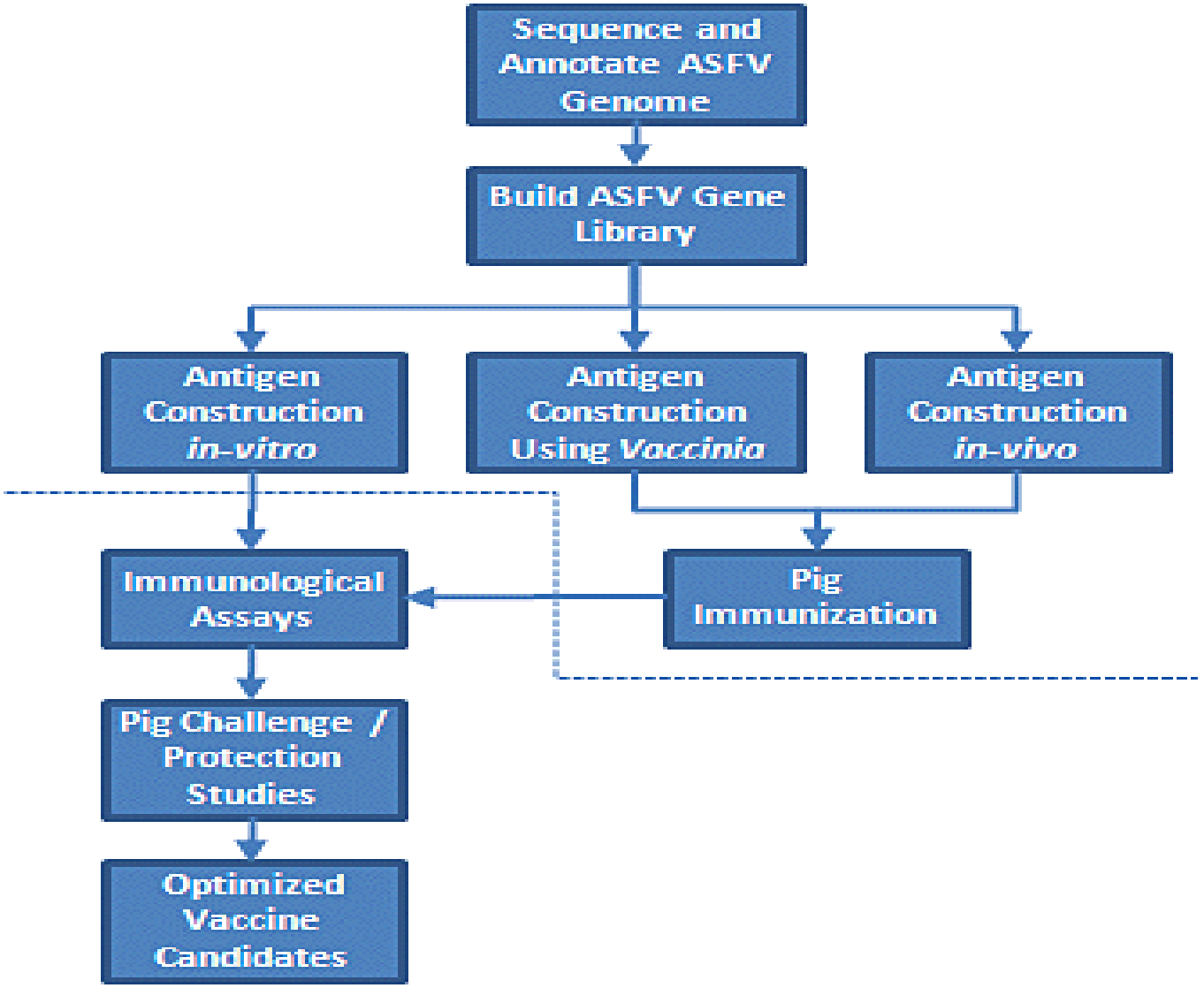


A238L



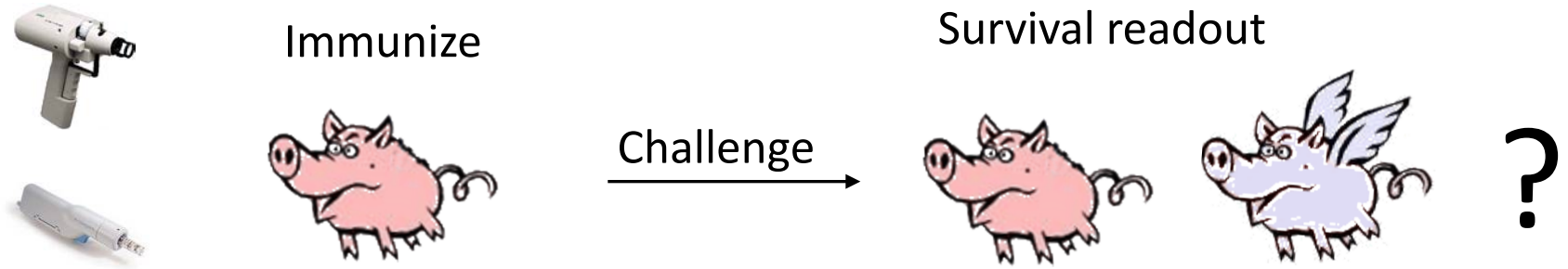
EP402R (CD2v)

Strategy for ASFV Library Construction



Challenge/protection experiments

1. Pool top antigens from each bin and immunize pigs with these pools of antigens by DNA prime and recombinant vaccinia virus boost.



2. Pool top 5-10 antigens from positive bins, and immunize pigs.
3. Re-test and validate vaccine candidates

Summary of Progress: genome wide antigen screen

- DNA vaccine and protein expression libraries complete
- rVV library 47 complete
- Immunome screening in pigs – conditions optimised and 47 antigens tested by DNA prime rVV boost
- T cell and antibody assays used to rank ORFs for immune responses
- First challenge experiments carried out

Future Prospects NGS

- Further complete ASFV genomes to expand spectrum of genotypes and phenotypically different viruses.
- RNA sequencing from cells or pigs infected with different ASFV isolates.

- better understanding of virus host interactions
- correlates of pathogenesis and protection

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