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# **Third Generation Sequencing Technologies to Decipher Genomic Structures of Recombinant-prone Viruses**



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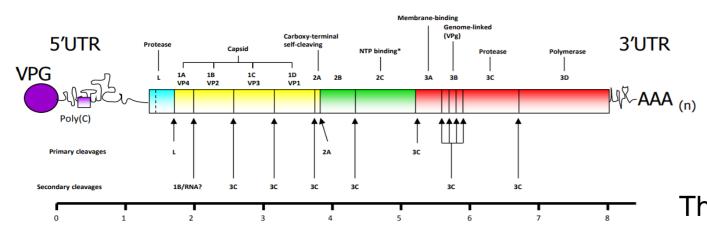
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# CONTEXT

Foot-and-Mouth Disease Virus : FMDV

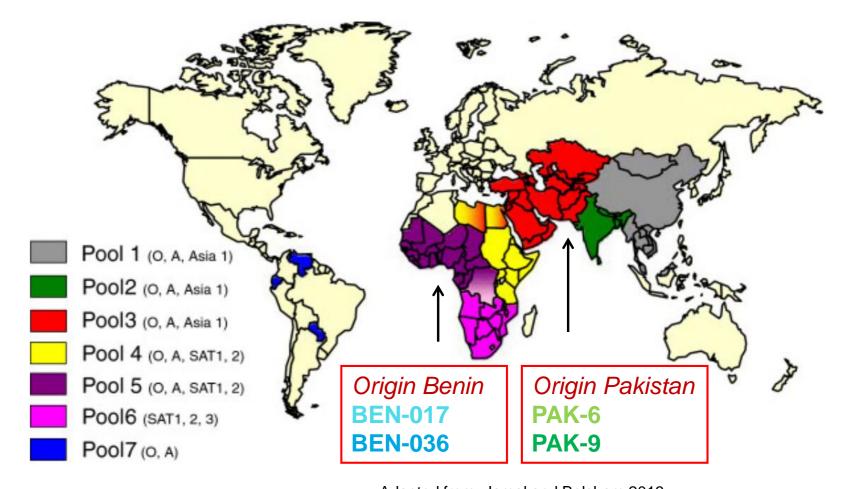
- > Family *Picornaviridae*, genus *Aphthovirus* ➢ RNA virus ~8300 nucleotides > 7 Serotypes (O; A; C; SAT1; 2; 3 and Asia-1)
- Prone to recombination



What is the interest in sequencing FMDV?

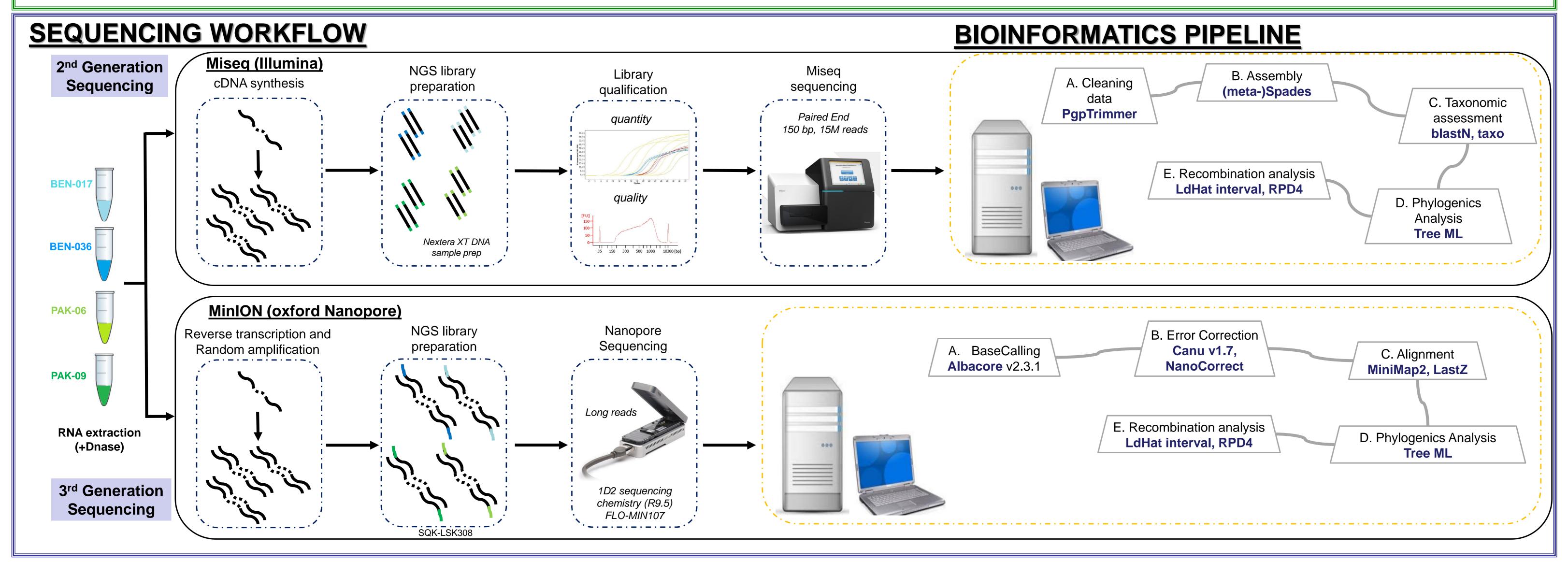
- Multiple virus serotypes/strains
- Monitoring global patterns of virus distribution
- Early recognition of the emergence of new lineages
- Antigenic prediction and vaccine selection
- Social economic impact of outbreak
- FMDV causes a highly contagious disease of clovenhoofed animals (cattle, goats, sheep, pigs ...).

The generation of recombinant viruses in the field has been reported earlier and such events have occurred within the coding region for the non-structural proteins (NSP) in the 3' portion of the genome and even in the capsid coding region of the genome. Four Isolates in our study



Kilobases

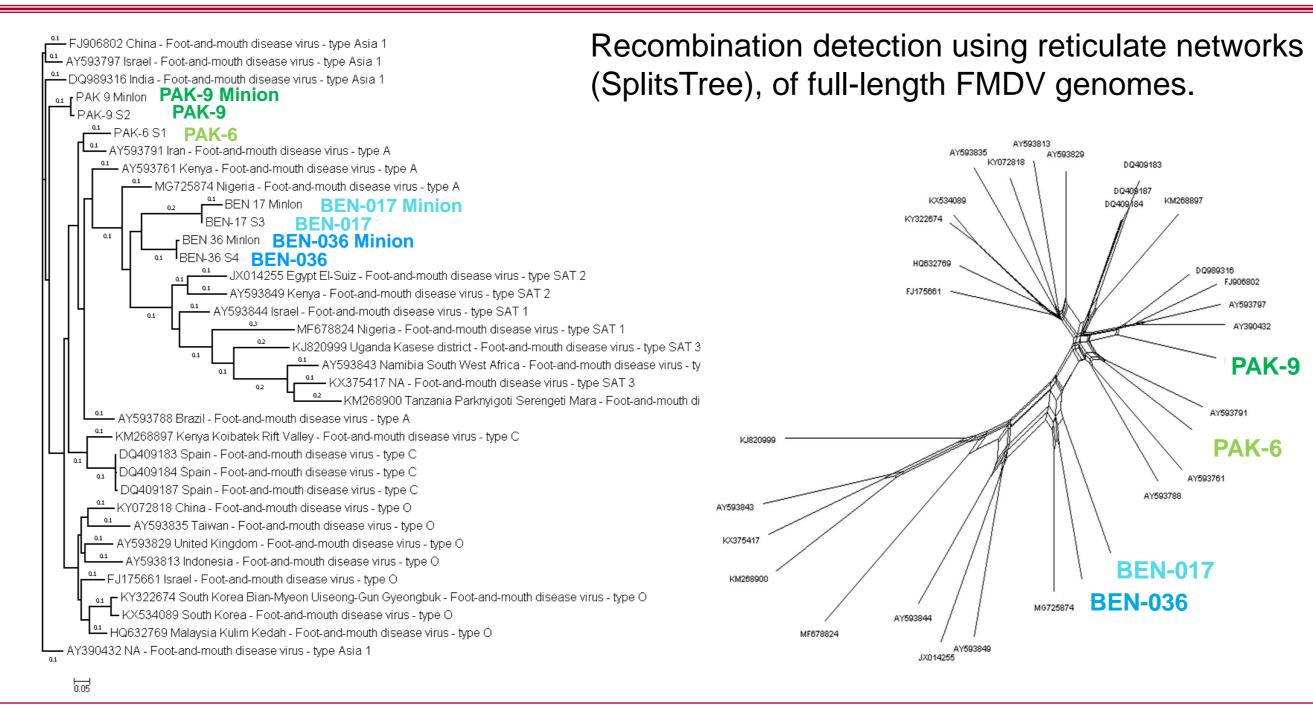
Adapted from: Jamai and Beisnam 2013

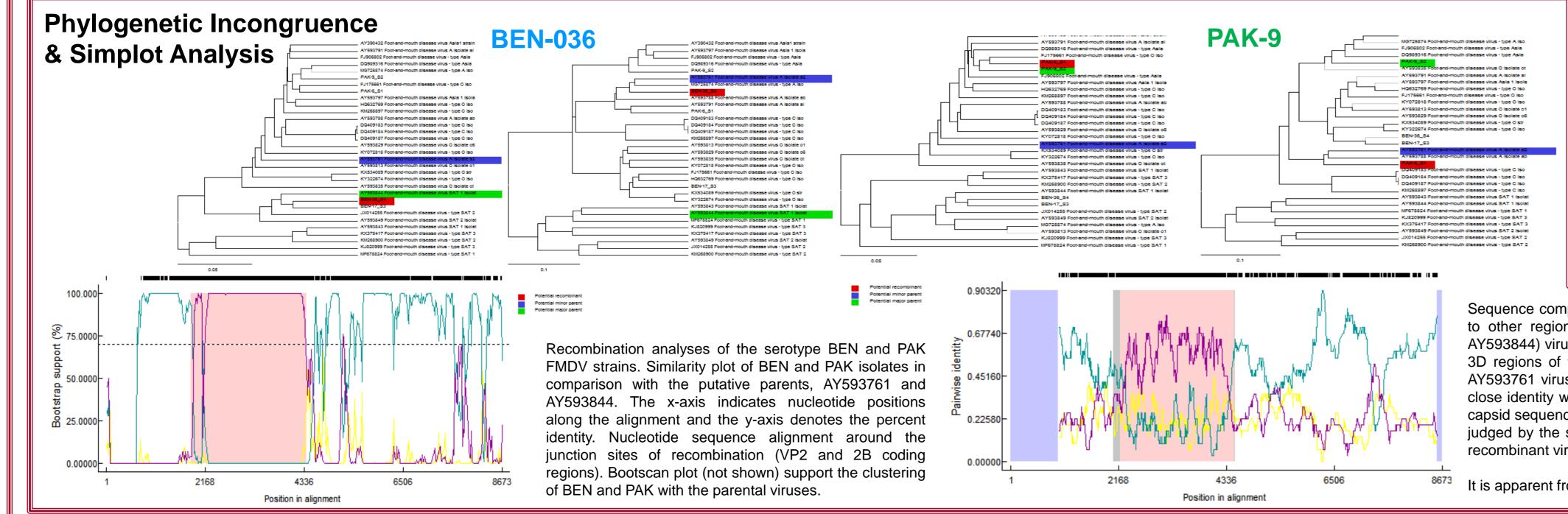


## PHYLOGENETIC ANALYSIS AND RECOMBINATION DETECTION

**Recombination region count matrix and recombination** region hot/cold spot matrix.

- Gathered 427 sequences from NCBI (may 2017)
- Clustered sequences by 86% into 28 clusters (4 Asia, 4 type C, 8 type O, 3 SAT 1, 3 SAT 2, 2 SAT 3, 4 type A).
- Add 4 newly sequenced isolates
- Progressive alignment was done using MAFFT (G-INS-i strategy, Iterative refinement method with global pairwise alignment.
- Fasttree ML tree (GTR model).
- **Recombination detection** 
  - LDHat interval analysis
  - RDP4 (infer breakpoint positions for every detected potential recombination event).

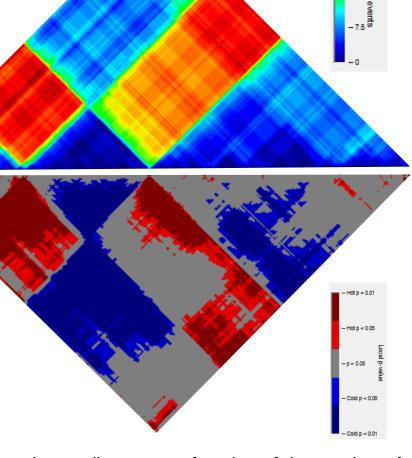




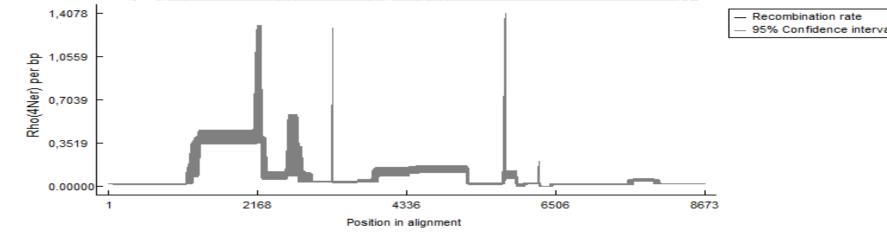
Mapping of the positions of recombination using phylogeny tree scanning recording the order of each variant in an alignment, giving a successive series of phylogenetic trees (rooted NJ trees, 100 bootstrap replicates, and where all branches with <70% support are collapsed, moving windows of 300nt and intervals of 100nt). The positions in the alignment where phylogenetic relationships change are examined and are of key interest.

AY593797

**PAK-9** 



Upper matrix: color gradients are a function of the number times that pairs of nucleotides were separated by observable recombination events. Lower matrix: pairs of sites that are the least (blue) and most (red) separable by recombination.



Variable recombination rate across the FMDV genomes. The black plot represents average estimate of point recombination rates determined by Reversible-Jump MCMC approach implemented in the interval component of LdHat. Gray region represents 95% credibility interval of point recombination in the RJMCMC chain.

The results of this analysis are in agreement with the phylogeneticcompatibility analysis in that the distribution of observed breakpoints appears to be non-random.

Sequence comparisons showed greater divergence from the BEN and PAK isolate sequences within the capsid coding sequence to other regions, indicating that these viruses could have been generated by recombination between a serotype (close to AY593844) virus and another virus serotype (close to AY593761). As is evident from the similarity plots, the 5'UTR, L, VP4, 3C and 3D regions of the BEN and PAK virus have very close nucleotide sequence identities with serotypes close to AY593844 and AY593761 viruses whereas, in contrast, the contiguous VP2, VP3, VP1 and 2A coding region of the BEN and PAK RNA showed close identity with the AY593844 and AY593761 sequences (consistent with the serotype designation which is determined by the capsid sequences). From the SimPlot analysis, it appears that the junctions for the recombination events between these viruses, as judged by the sharp switch in level of sequence identity, are close to nt 2168 and nt 4336. It would seem likely that these two recombinant viruses are the progeny of a similar recombination event.

It is apparent from this work that at least three different FMDV serotype groups have been circulating in Pakistan and Benin.

### **CONCLUSION**

- Both sequencing technologies allowed the characterization of the full genome for the 4 isolates. The serotype of each sample was correctly assigned.
- Extended comparison of sequences including all available complete genome sequences available from public databases allows to explore differences in the frequency of recombination between isolates.
- Exhaustive comparison highlights increased basal recombination rates across the genome, maps regions of phylogenetic incongruity and infers regions of favored recombination.
- Results of these FMDV breakpoint distribution and phylogenetic-compatibility analyses reflect a clear partitioning of structural and non-structural genes in the organization of the genome.
- → Genome organization facilitates component swapping or recombination that frequently occurs among such viruses. Confident construction of transmission trees from phylogenetic data, using MinION nanopore sequencing, offers an exciting potential to FMDV diagnostics, and more specifically for resolving recombination scenarios. Such approaches, integrating both novel technological sequencing instruments, together with phylogenetic and epidemiological data, will help understand mechanisms involving the recombination patterns observed in FMDV and other picornaviruses, and will eventually lead to novel insights into epidemiological and phylogeographics issues in FMDV outbreaks.

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