

PCR-sequencing of the BVDV genome reflects quasi-species variation defined by next-generation sequencing

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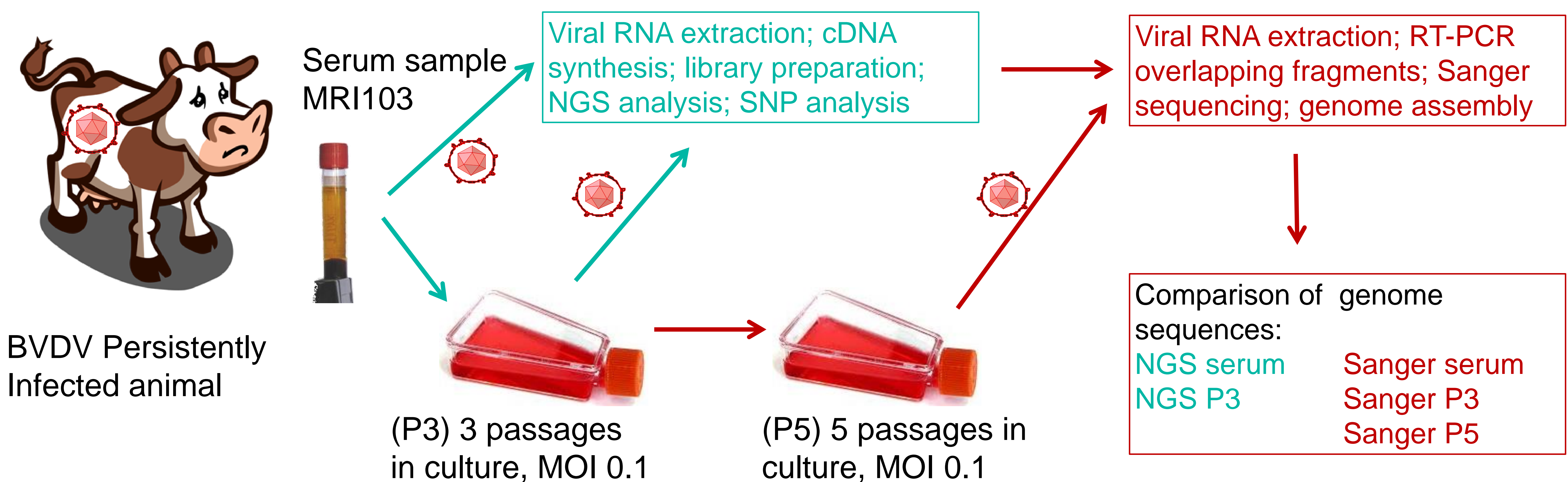
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Introduction

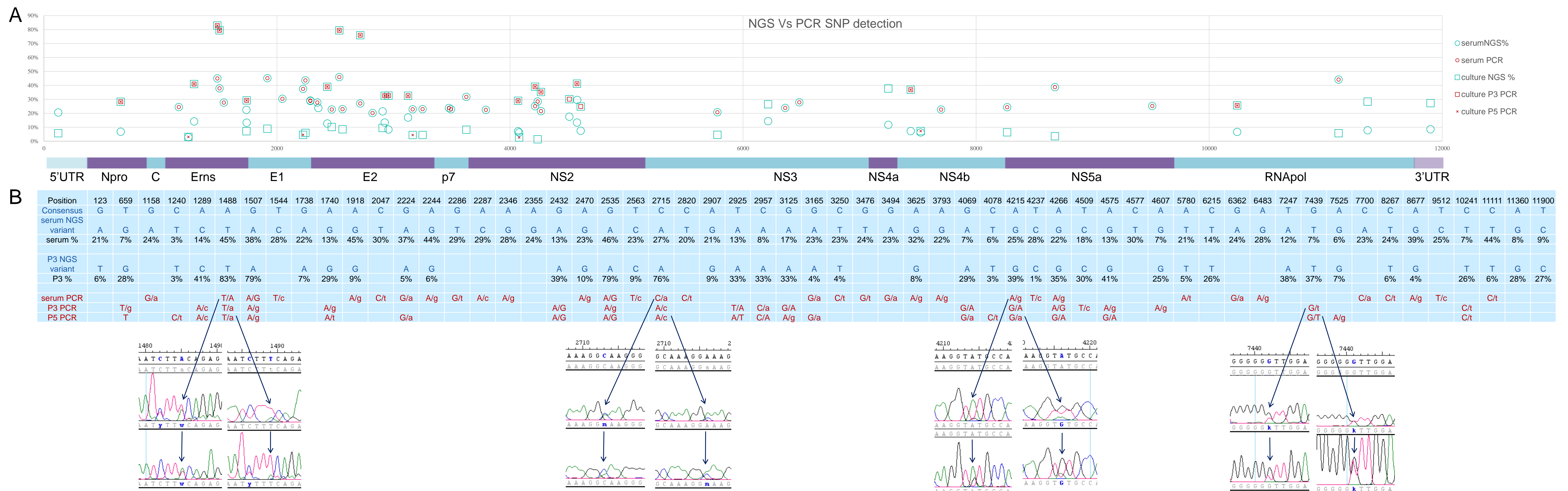
Analysis of RNA virus genomes can be complicated by their error-prone replication, which produces a population of closely related viral sequences in infected individuals. While next-generation sequencing (NGS) allows the quasi-species nature of RNA virus infection to be elucidated, this can be expensive in relation to genome size or require large amounts of material. To study Bovine Viral Diarrhoea Virus (BVDV) sequence variation, we have developed methods to amplify segments of the BVDV genome from very small diagnostic samples. Here we describe a comparison of NGS sequencing with virus-specific RT-PCR and Sanger sequencing, using BVDV from the serum of a persistently-infected animal and the same virus after passage in culture. NGS analysis of the virus BVDV-MRI103 showed that 276 positions within the assembled BVDV genome sequences were polymorphic in one or both genome assemblies, while the consensus sequence of the cultured virus differed at only 4 positions from the virus in serum.

To develop a generic approach to sequencing BVDV genomes from virus-positive diagnostic samples, primers were designed to amplify 24 PCR products that spanned the entire BVDV genome with a high degree of overlap. While the consensus sequences produced by the two approaches were essentially identical, the chromatogram traces of the PCR-derived sequences also contained evidence of many of the SNP detected by next generation sequencing.

METHODS:



RESULTS:



Sequencing of BVDV samples by NGS and PCR-Sanger approaches. NGS sequencing of the BVDV strain MRI103 was done by TGAC by Illumina short-read technology on unamplified cDNA produced from viral RNA from serum or from passage 3 (P3) of culture. PCR-sequencing used RNA purified from the same material (serum and P3) and from P5 cultured virus, amplifying 24 overlapping fragments using primers based on the genomic sequence. (A) Variant sites in the NGS data were scored by read-count as non-consensus base calls represented in >5% of reads, while PCR sequence variants were those with clear trace data for multiple base calls in at least two reads at the identified position. Four examples of trace data representing polymorphic differences between the serum virus and P5 cultured virus are shown.

SUMMARY:

- Essentially complete BVDV genome sequences were obtained from overlapping PCR products
- Sanger sequence data identified sites of quasi-species polymorphism within the BVDV genome
- Virus consensus sequence changed even after 3 culture passages
- P5 culture fixed some SNPs that were very low frequency in the serum virus

ACKNOWLEDGEMENTS:

