CHAPTER 5

Concluding remarks: What does the future hold?

Custom-made vaccines, produced in response to specific outbreaks of FMD, may be achieved through the construction of recombinant viruses. In such recombinants, the antigenic determinants of a stable, high antigen producing and well-characterized FMDV strain may be manipulated to mimic the antigenicity of outbreak strains, leading to the production of conventional, but custom-made vaccines. Although a number of crucial factors, which may influence the success of such a strategy, were addressed in the current study, there are still many questions in need of answering. These include the protective roles of conformational and non-conformational epitopes as well as the role of proteolytic variation and cleavage site differences in isolates from different geographical localities.

Following the study on the genetic heterogeneity of the L^{pro} and $3C^{pro}$ of FMDV, it was seen that these genomic regions of the SAT type viruses originating from southern Africa, are distinct from that of types A, O and C. Although the three-dimensional structure of the ZIM/7/83/2 Lb^{pro} is completely conserved upon comparison to the Lb^{pro} crystal structure of O_1Kaufbeuren, the differences detected on the L/P1 cleavage site could have implications for intertypic processing. These implications (if any) will subsequently be investigated with the expression of Leader proteinases of SAT type representatives in bacteria. The purified proteinases will be assayed on oligopeptides corresponding to the cleavage site of the Leader proteinases of the European types. The activity of the proteinases to cleave the host cell protein eIF4G will also be examined. These biological assays might shed some light on the flexibility of FMDV proteolytic activity.

Despite the unknown factors, an infectious chimeric or recombinant virus between types A and SAT 2 was successfully constructed. This recombinant virus (#14A12/SAT2) contains the genetic background of type A_{12} as well as the external capsid region of ZIM/7/83/2. The subsequent evaluation of #14A12/SAT2 with respect

to the wild type SAT 2 virus revealed the chimera not only to be less stable, but also exhibited a slower growth rate. These characteristics make the current recombinant virus unsuitable for commercial vaccine manufacturing. Alternative means should therefore be explored to be able to produce stable and high antigen producing chimeric SAT viruses in order to address the antigenic variation in these viruses and the subsequent problems associated with vaccine production.

One such alternative option entails the construction of a full-length cDNA clone suitable for the southern African SAT type viruses. Such a cDNA clone has been constructed for the SAT 2 virus, ZIM/7/83/2 (Mason & van Rensburg, unpublished results). An exchange-cassette strategy (Figure 5.1) for the construction of this clone was followed. This approach entailed the exchange of certain regions of the original A_{12} full-length cDNA clone with the corresponding regions on the SAT 2 genome. These regions were obtained through PCR amplification from cDNA using a high fidelity enzyme and oligonucleotides, specifically designed with the required restriction enzyme sites to facilitate the cloning. During the construction of the cDNA clone, the 5' UTR was molecularly characterized. Despite low nucleotide sequence identity in this region (including a 37 nucleotide deletion in the S-fragment obtained for the SAT 2) between A_{12} and ZIM/7/83/2, similar folded structures could be obtained for the four pseudoknots and the S-fragments (hairpin-structure) of these viruses (results not shown).

Through the subsequent evaluation of these chimeric viruses (Figure 5.1) and comparison to the wild type SAT 2 virus, indications with regard to regions on the genome responsible for the reduced growth properties and lack of stability, might be obtained. The SAT 2 full-length cDNA clone will also be used to construct SAT 1 and SAT 3 chimeric viruses and together with similar constructs employing the A_{12} cDNA clone, be evaluated in terms of growth properties and stability. Such a strategy will, hopefully reveal whether the SAT 2 replicating cDNA clone is indeed a viable alternative in the construction and production of SAT chimeric viruses.

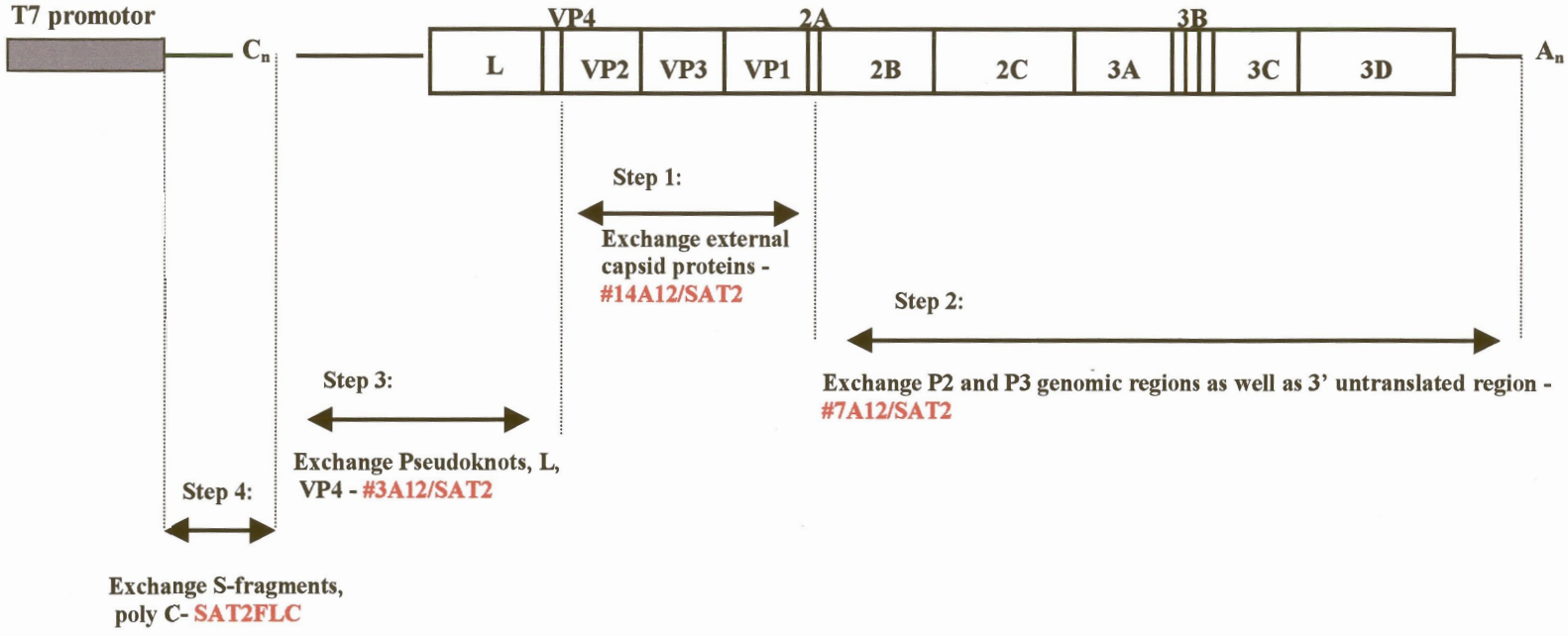

Figure 5.1: Schematic representation of the construction of the SAT 2 full-length cDNA clone (SAT2FLC). The chimeric SAT viruses obtained