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Poster Abstract – F.12

RISK AND STABILITY ASSESSMENT OF A POTATO VIRUS X-BASED VECTOR FOR RECOMBINANT PROTEIN EXPRESSION

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We investigated the stability of expression constructs based on Potato Virus X (PVX) as a function of insert length. Five different inserts ranging in length from 261 to 1758 bp (human proinsulin, murine interleukin-10, HIV-1 *nef*, petunia expansin-1 and human *GAD65*) were expressed using a PVX vector in *Nicotiana benthamiana* plants for three sequential passages. Using a competitive RT-PCR approach we demonstrated that all five inserts could be deleted in the first infection cycle, but that this was much more likely to occur for longer inserts. This suggested a negative correlation between insert length and vector stability. Sequence analysis of the deleted constructs suggested that recombination usually occurred at sites close to the duplicated subgenomic promoter, but in a smaller number of cases the foreign gene itself was probably involved resulting in partially deleted constructs containing transgene fragments. The implications of these results in the context of viral replication and the risk assessment of expression vectors based on plant viruses are discussed.