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Cloning a defective SIVmac239 genome into modified vaccinia Ankara (MVA)

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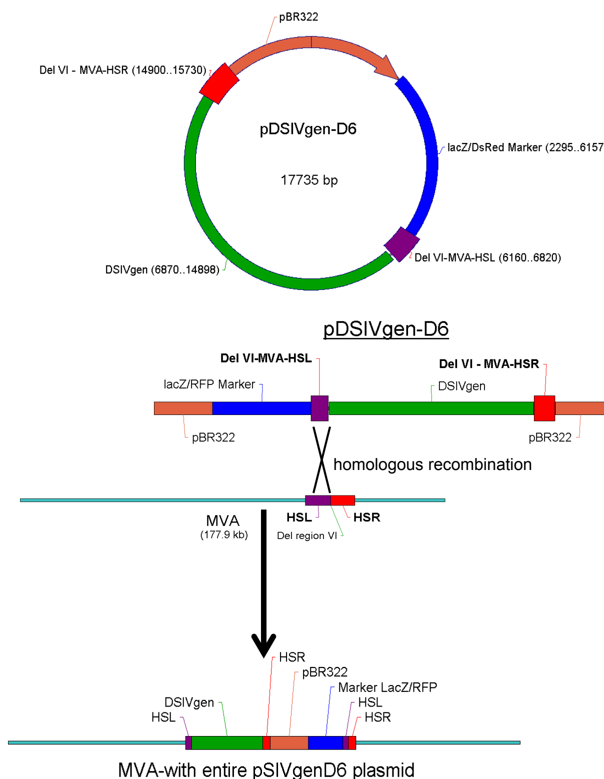
Background

It has previously been shown that MVA has a cytokine receptor profile and replication properties that make it a promising recombinant HIV vaccine vector. Recombinant MVA boosts T cell responses very efficiently and is currently widely used as vaccine candidate for many pathogens including HIV. Attempts to achieve a similar effect in eliciting neutralising antibodies to primary isolates of HIV employing MVA expressing HIV virus-like particles (gag & env) have been disappointing so far. Here we describe a method for inserting the genome of an HIV-like virus within the genome of MVA. The first naturally-occurring example of a poxvirus encoding an infectious retrovirus was discovered by Hertig et al. We have mimicked this natural recombination event by employing a synthetically constructed defective SIVmac239 genome recombined within a modified vaccinia Ankara (MVA) genome driven by T7 RNA polymerase.

Methods

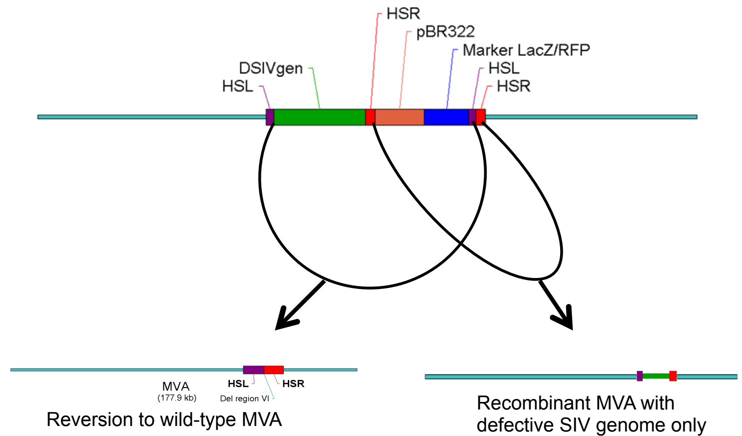
Our recombination vectors employ transient β-galactosidase (β-gal) and red fluorescent protein (RFP) markers, enabling the construction of complex markerless recombinants in primary chick embryo fibroblasts. The defective SIV genomic plasmid employed for recombination was 17,735 base pairs in size, inserting in deletion VI of the MVA genome. Multiple mutations and deletions introduced in the defective SIV genome prevent the formation of infectious retroviruses by vertebrate cells infected by recombinant MVA, but limited production of retroviral proteins is predicted to occur. The defective SIV genome encodes green fluorescent protein adjacent to an internal ribosomal entry site, to enable easy detection of retroviral RNA

Map of recombination plasmid showing defective SIV genome



MVA-with entire pSIVgenD6 plasmid

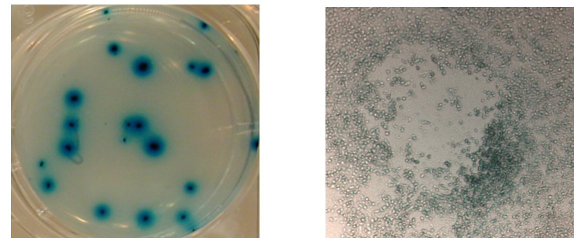
Second intragenomic recombination resulting in loss of markers



Reversion to wild-type MVA

Recombinant MVA with defective SIV genome only

Plaques of recombinant MVA encoding defective SIV genome and marker genes



Results

The defective SIVmac239 genome was synthesized in tandem with selection and recombination sequences, and all cloned into a pBR322 plasmid then sequenced. A separate similar plasmid encoding phage T7 RNA polymerase with a different MVA insertion site was also constructed and sequenced. Coinfection (with MVA) and cotransfection (with plasmids) of primary chick embryo fibroblasts demonstrated expression of β-gal but not RFP. Construction of recombinant MVA encoding SIVmac239 was accomplished by selection for β-gal alone.

Conclusions

The construction of recombinant MVA encoding a synthetic HIV-like genome is described. In distinction from earlier work, only transient expression of markers is required, enabling the future construction of more complex recombinants.

Acknowledgements

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References

Hertig, C., et al.Virology, 1997. **235**(2): p. 367