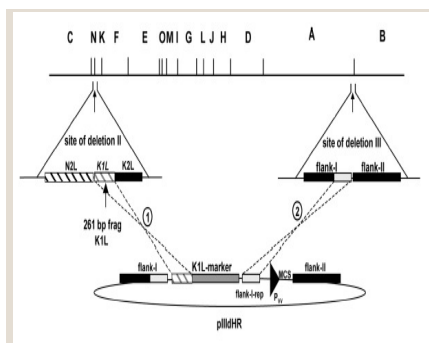


Challenge

Absence of pathogenicity for humans, avirulence even in immunocompromized hosts, high-level expression of foreign antigens and strong adjuvant effect make recombinant MVA (rMVA) an ideal vector for both prophylactic and therapeutic vaccination. The generation of rMVA relies on in vivo homologous recombination, a low-frequency event. rMVA must be purified from a large excess of parental non-recombinant MVA. Such isolation can be improved by so-called host-range selection systems, enabling recovery of rMVA by selective growth on certain cell lines.

The MVA host range gene and K1L-orthologue ORF 022L, which is mostly deleted in MVA, can be used for host range selection of rMVA. By introduction of the K1L gene along with a gene of interest, growth of MVA on the rabbit kidney cell line RK-13 is reconstituted. rMVA is rescued and subsequent plaque purified on RK-13 cells. After successful isolation, the K1L gene can be deleted by culture of rMVA on non-selective growth conditions, e.g. on CEF, leading to loss of the K1L gene.

Technology



Possible pitfalls of conventional K1L-selection

Source: Staib et al., BioTechniques

So far, a significant part of rMVA generated by K1L-selection was not expressing the foreign gene, but was able to grow on RK13 cells. This is due to the presence of a residual 263 bp fragment of the K1L gene in the MVA genome. Therefore, after introduction of a host range selection plasmid carrying a K1L-gene and a gene of interest, such gene can be deleted by a second homologous recombination event between the vector K1L gene and the residual K1L-sequences, resulting in a rMVA, which grows on RK13 cells, but does not express the foreign gene (see figure). Therefore, MVA lacking the residual K1L-sequences ("MVA-II-new") can be used for selection of rMVA, with a success rate of almost 100%.

Commercial Benefit and Opportunity

The novel selection method allows the rapid identification and isolation of rMVA using an efficient host range selection system. Thus, the improved method already allowed us to obtain new important rMVA vector constructs which if to be generated by standard procedures would have been more cumbersome if not impossible.

The technology is available for non-exclusive licensing. Parties interested in collaborative research and development are highly welcomed.

Developmental Status

The described method is currently used as standard selection for rMVA in the lab.

Patent Situation

A European patent has been granted (EP1594970), patent applications are pending in CN, JP, SG and US.

Relevant Publication

Staib et al. (2004), *Methods Mol. Biol.* 269, 77-100; Staib et al. (2003), *BioTechniques* 34, 694-700.

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