# Construction of recombinant ORFV-TRAP using CRISPR/Cas9 technology

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### Abstract

Orf is an acute and highly contracted human and animal infection caused by orf virus (ORFV), which mainly affects sheep and goats, especially young animals, who have close contact with sick animals. Clinically, in secondary infection cases of orf, opportunistic or conditional pathogens such as Staphylococcus aureus (S. aureus) are often detected. The S. aureus TRAP gene product is reportedly protective against bacterial infection. For joint control of ORFV and S. aureus, the research direction of delivering a TRAP gene vaccine against S. aureus infection using the ORFV live vector is proposed. Here, we used CRISPR/Cas9 technology to edit vascular endothelial growth factor E of ORFV (VEGF-V) and introduced the TRAP gene of S. aureus into the terminus of the ORFV genome to promote TRAP expression in infected keratinocytes. The construction and experimental verification of recombinant ORFV (ORFV-V/TRAP) will provide a reference for in-depth studies on the prevention and control of orf and S. aureus disease.

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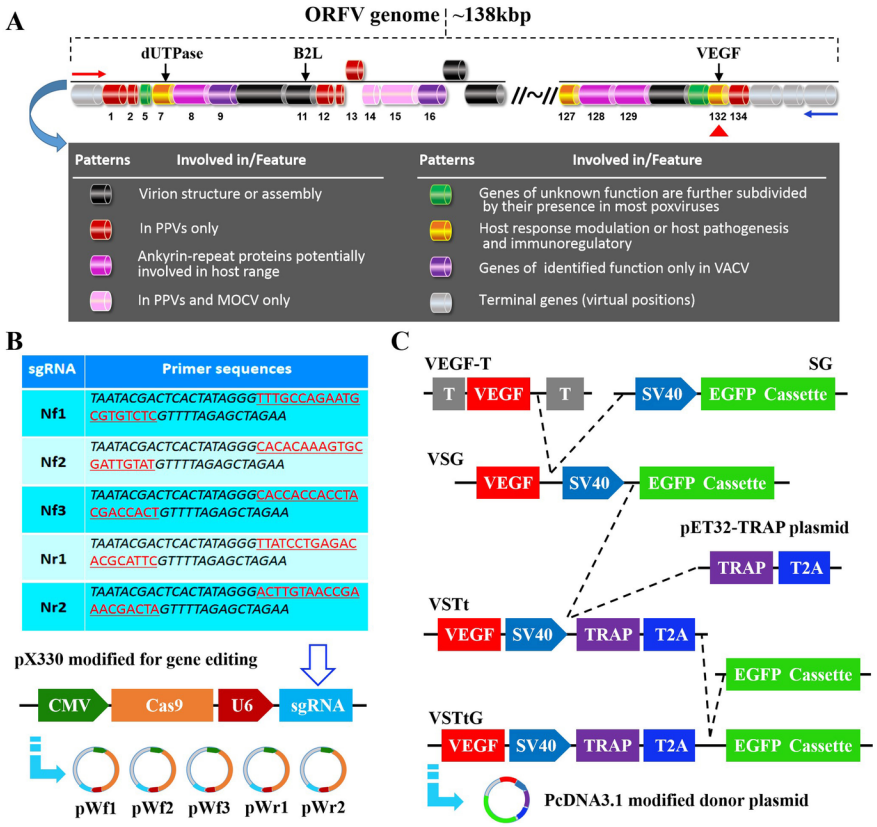
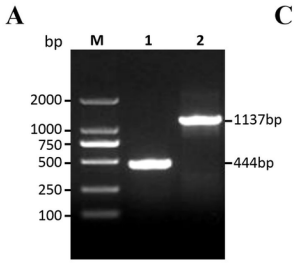
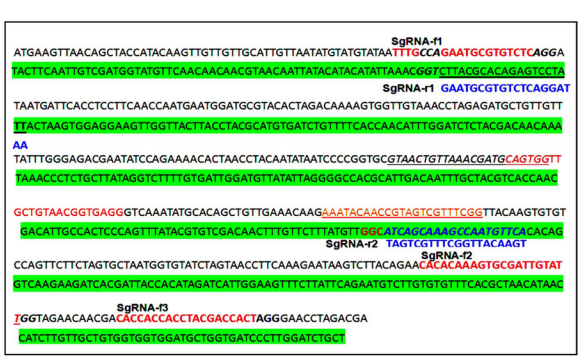



B

Putative amino acid sequence of VEGF

MKLTATIQVVVALLICMYNLPECVSQD
NDSPPSTNEWMRTLDKSGCKPRDAV
VYLGDEYPENTNLQYNPRCVTVKRCS
GCCNGEGQICTAVETRNTTVVVSVTS
VSSSSSANGVSSNLQRISLTEHTKCDCI
GRTTTPPPTTTREPRR-**148aa**




D

| SgRNA name | Nf1 | Nr1 | Nr2 | Nf2 | Nf3 |
|---|---|---|---|---|---|
| Position in VEGF sequence | 56 | 63 | 292 | 375 | 409 |