Using Recombination-Dependent Lethal Mutations to Stabilize Reporter Flaviviruses

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Introduction
Flaviviruses, including the recently emerged Zika virus (ZIKV) and the prototypical yellow fever virus (YFV) are significant human pathogens. Engineering these viruses with reporter genes allows for greater throughput in pathogenesis studies, antiviral assays, and sero-diagnostic assays. Past flavivirus reporter constructs have been thwarted by recombination-driven genetic instability.

Reporter flavivirus utility is limited by genetic instability during passaging.

Methods
Flaviviruses require a threshold of positive charges in the beginning of the capsid gene for virion formation. Positive to negative mutations in the duplicated capsid (C25) become lethal for viral particle formation upon recombination.

- Positive to negative charge mutations were screened in the capsid for NanoLuc-bearing ZIKV and YFV.
- These mutations were then engineered in C25 of ZIKV (DK23 Nano) and YFV (YF4 Nano) and the viruses were characterized.
- Reporter viruses were passaged in duplicate in Vero cells ten times. Samples from each passage were assayed by RT-PCR and gel electrophoresis for stability.

Results

Scheme of recombination-dependent lethal mutations

A

<table>
<thead>
<tr>
<th>5' UTR</th>
<th>C25</th>
<th>P2A</th>
<th>NanoLuc</th>
<th>C25</th>
<th>Capaid</th>
</tr>
</thead>
</table>
B

| 5' UTR | C25 | P2A | NanoLuc | C25 | Capaid |

Growth characteristics of C25 mutated ZIKV (DK23 Nano) and YFV (YF4 Nano) virus compared to non-mutated reporter virus and WT parental virus.

Conclusions
While recombination-dependent lethal mutations have a slight attenuating effect on viral replication, they are effective in stabilizing the NanoLuc gene for ten passages. This strategy overcomes previous shortcomings in the flavivirus field, allowing greater confidence in reporter virus assays, such as antiviral compound studies or reporter neutralization tests.