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Original modifications of peste des petits ruminants virus (PPRV) genome induced by RNA interference (RNAi)

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INTRODUCTION

Peste des petits ruminants virus (PPRV) is a morbillivirus responsible for a disease that affects sheep, goats and some small wild ruminants species.

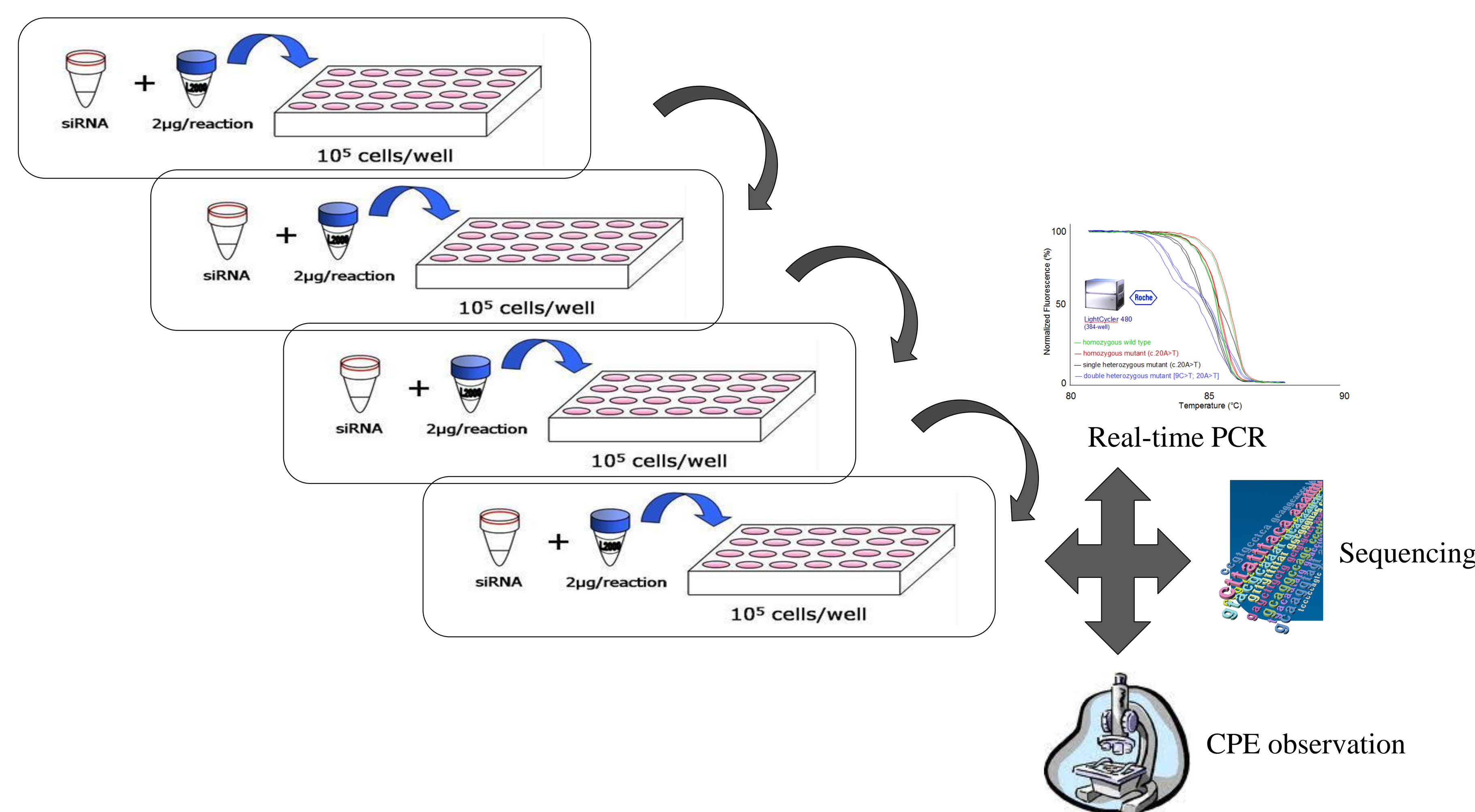
CIRAD previously identified three small interfering RNA that target conserved regions of the essential gene encoding the viral nucleoprotein and prevent at least 90% of PPRV replication *in vitro*. These molecules are currently under *in vivo* evaluation. However, there is an important risk of emergence of virus escape mutants since a single mutation can abrogate the RNA interference (RNAi).

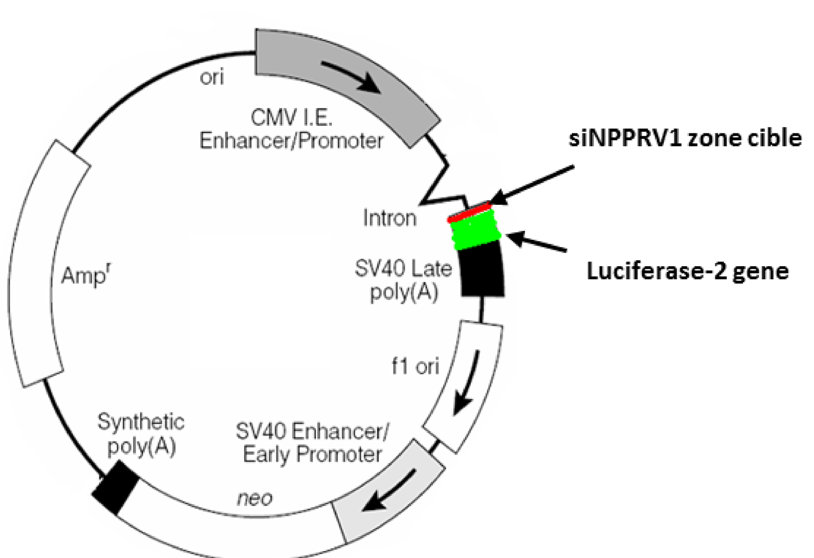
OBJECTIF

In this study, we investigated the ability of PPRV to escape the inhibition conferred by single or multiple siRNAs after several consecutive passages *in vitro* at suboptimal doses.

MATERIAL & METHODS

Vero cells were plated with different final concentrations of each siRNAs (100; 33.3; 11.1 and 3.7nM) complexed with Lipofectamine 2000 (Invitrogen). Twenty-four hours later, cells were infected with ten-fold dilutions of the virus collected in the previous transfection and the virus titration were realized. Four days later, the siRNA silencing effect was evaluated by scoring the reduction of CPE and the virus were collected, for to use in the next transfections and for the characterization of the mutations by sequencing of the N gene and for the quantification of the mutant and wild virus populations by real-time PCR.





The role of the detected mutations in escaping RNA interference was confirmed by using a reporter gene system based on the luciferase gene placed under the original or mutated target sequences. The *in vitro* luciferase activity is measured using IVIS-Lumina II (Caliper Life Sciences, USA) after addition of D-Luciferin potassium salt. The bioluminescence signal obtained was measured and quantified using the Livibng Image software version 4.0.

RESULTS

Except with the combination of the three different siRNAs, the virus systematically escaped RNAi after 3 to 20 consecutive passages (Table 1).

Mutations were characterized by single or multiple punctual nucleotide mutations (synonymous or not).

A deletion of a stretch of six nucleotides was also observed. While still complying with the so-called '*rule of six'* in the morbillivirus genome for optimized replication, this deletion was shifted in the open reading frame (ORF). However, the shift only resulted in the loss of 2 amino-acids, the rest of the protein remaining unchanged.

	siNPPRV1	siNPPRV6	siNPPRV7
don't mutation	GGATCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT
si1test1	GGATCGGCTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT
si1test2	GGATAAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT
si1test3	GGATAAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT
si1test4	GGATCAGCTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT
si1test5	XXXXXXACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT
si6test1	GGATCAACTGGTTTGAGAA	GGCGGTTCACGGTATCTCT	GCATTAGGCCTTCACGAGT
si6test2	GGATCAACTGGTTTGAGAA	GGCGGTTTATGGTATCTCT	GCATTAGGCCTTCACGAGT
si6test3	GGATCAACTGGTTTGAGAA	GGCGGTTTATGGTATCTCT	GCATTAGGCCTTCACGAGT
si7test1	GGATCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGGCTTCACGAGT
si7test2	GGATCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGGCTTCACGAGT
si7test3	GGATCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGGCTTCACGAGT
si7test4	GGATCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGGCTTCACGAGT
si7test5	GGATCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGCCCTTCACGAGT
si1et7	GGATCAATTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGGCTTCACGAGT
si1et6	GGGTCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT
si6et7	GGATCAACTGGTTTGAGAA	GGCGGTTTATGGTATCTCT	GCATTAGGGCTTCACGAGT
si1et6et7	GGATCAACTGGTTTGAGAA	GGCGGTTCATGGTATCTCT	GCATTAGGCCTTCACGAGT

Table 1: Mutations and one deletion that resulted from consecutive transfections *in vitro*

By using a reporter plasmid based on the luciferase gene, all mutations and the deletion obtained by transfections with the siNPPRV1 were confirmed as directly responsible for the viral escape (Figure 1). Off-target effects were not evidenced.

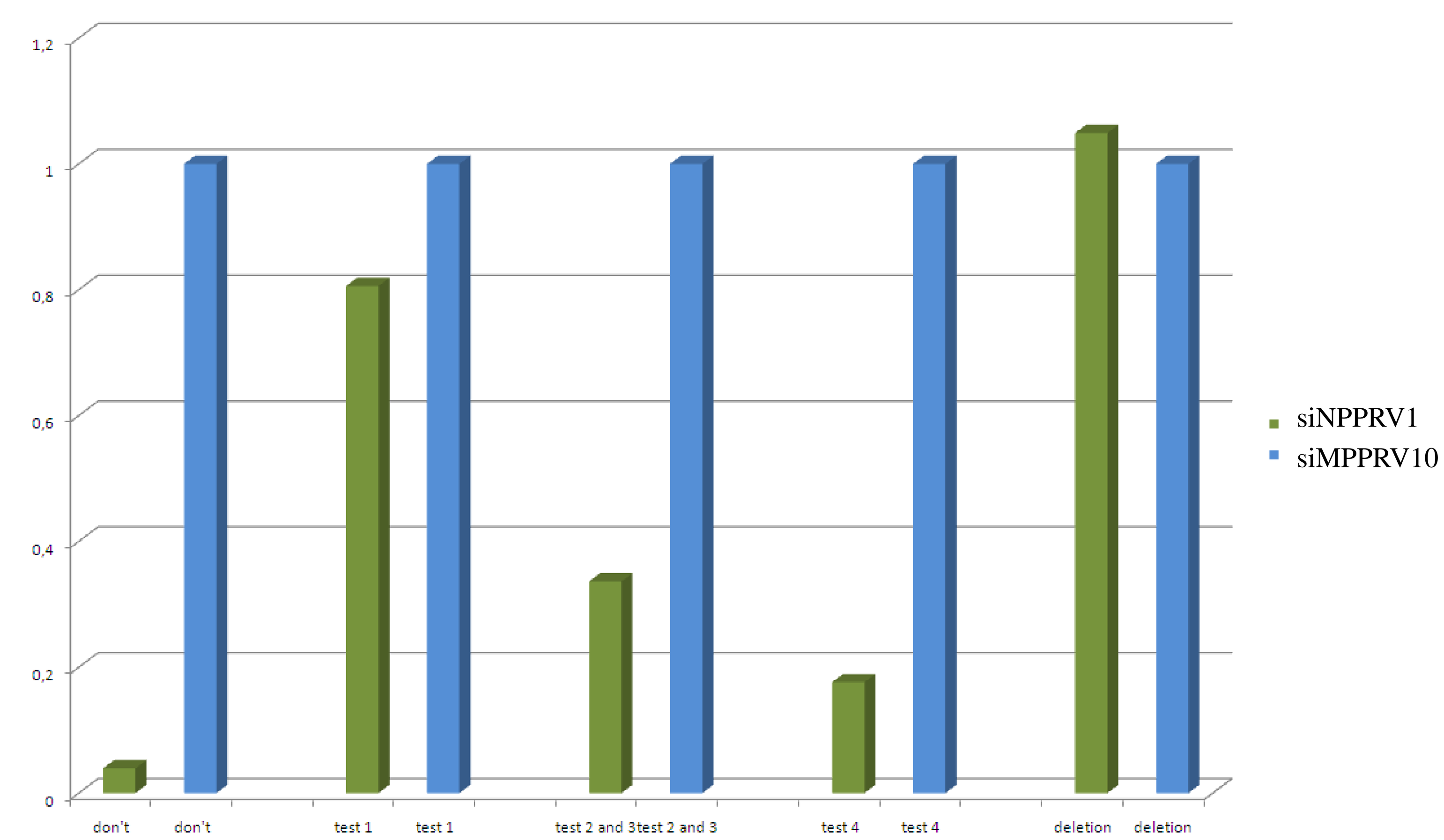


Figure 1: Comparison of the bioluminescence signal between the reporter plasmid with the original sequence of the siNPPRV1 and the plasmids with the siNPPRV1 mutated or deleted sequences. For the control, a siRNA irrelevant (siMPPRV10) targeting the M protein was used.

CONCLUSIONS

Several mutations were generated in the targets of our siRNA, allowing the virus to escape RNA interference. Sequence deletion often occurs when a non coding or non essential gene is targeted. However, in this work we were able to find for the first time an escape mutant with a deletion in an essential and highly conserved viral gene resulting in the production of a shorter protein.

This study provides new insights on the genomic plasticity of morbilliviruses that should be considered in antiviral strategies.

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