



Engineering the salamander genome using CRISPR -Cas9 triggered genomic tailoring

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Introduction

Why Salamanders?

It is important to study regenerative capacity of salamanders at the molecular level as this will enhance our knowledge to induce same effect in other non-regenerative models, with an ultimate goal to apply to *Homo sapiens*. To make it possible it is not only important to study animal models at the genetic level, but also to efficiently tailor their genes.

CRISPR-Cas9 as Engineering Tool

Till now clustered regularly interspaced short palindromic repeat (CRISPR) method has proved to be a powerful tool to manipulate genetic signaling in various species covering from *C.elegans* to *in vitro* human cell lines.

We are using CRISPR-Cas9 system to manipulate salamander's genetic circuit, suspecting to play a role in regeneration.

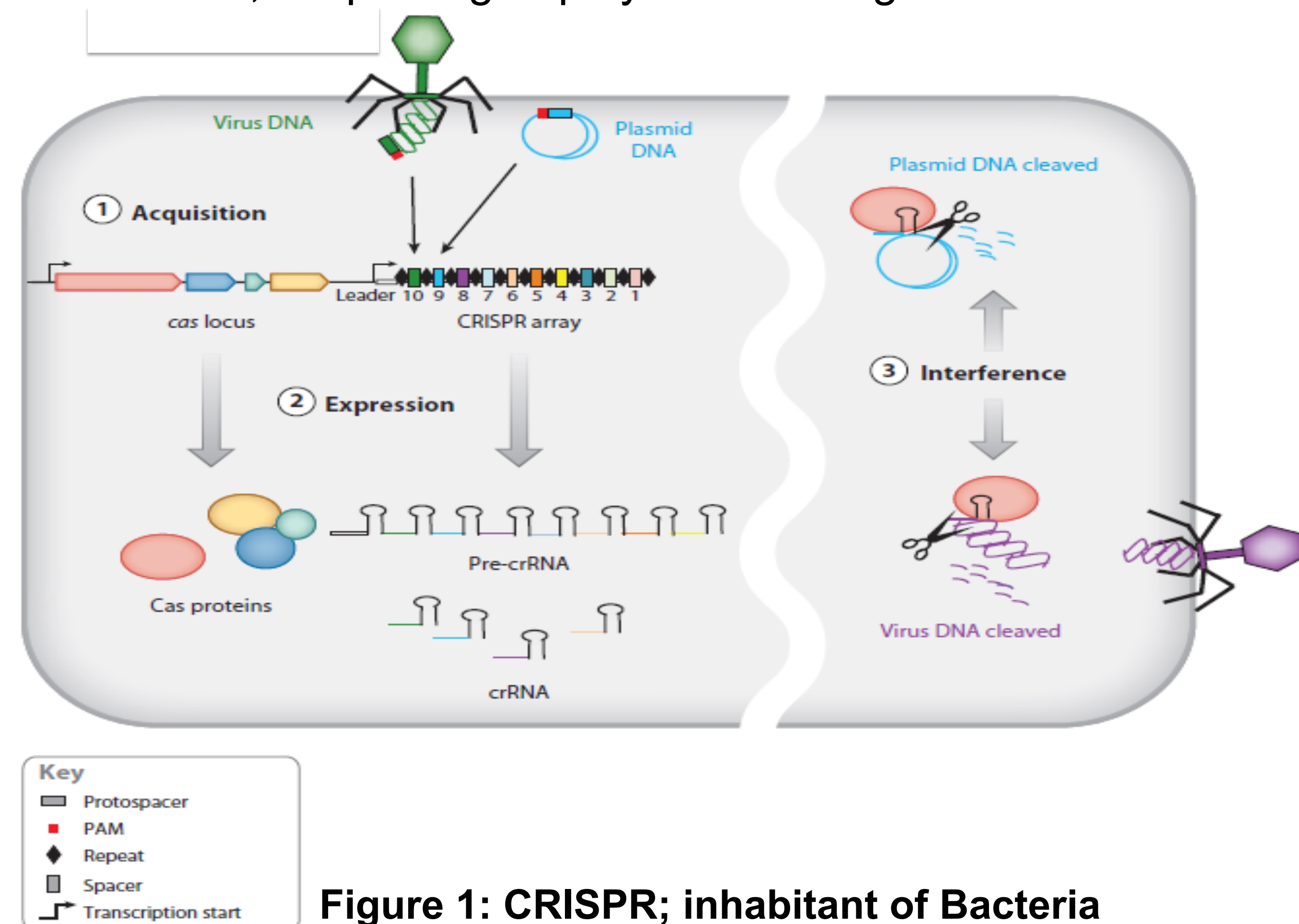


Figure 1: CRISPR; inhabitant of Bacteria

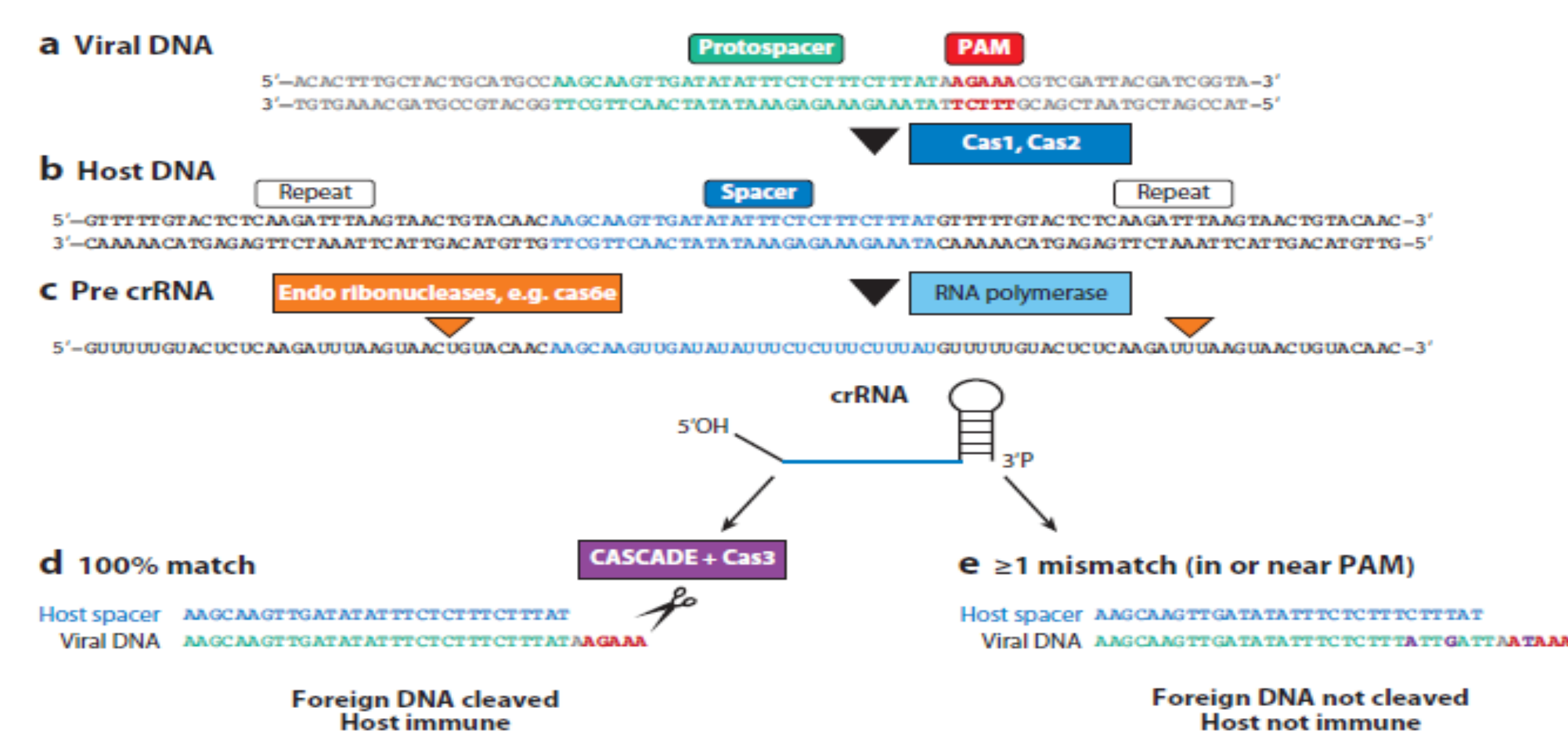
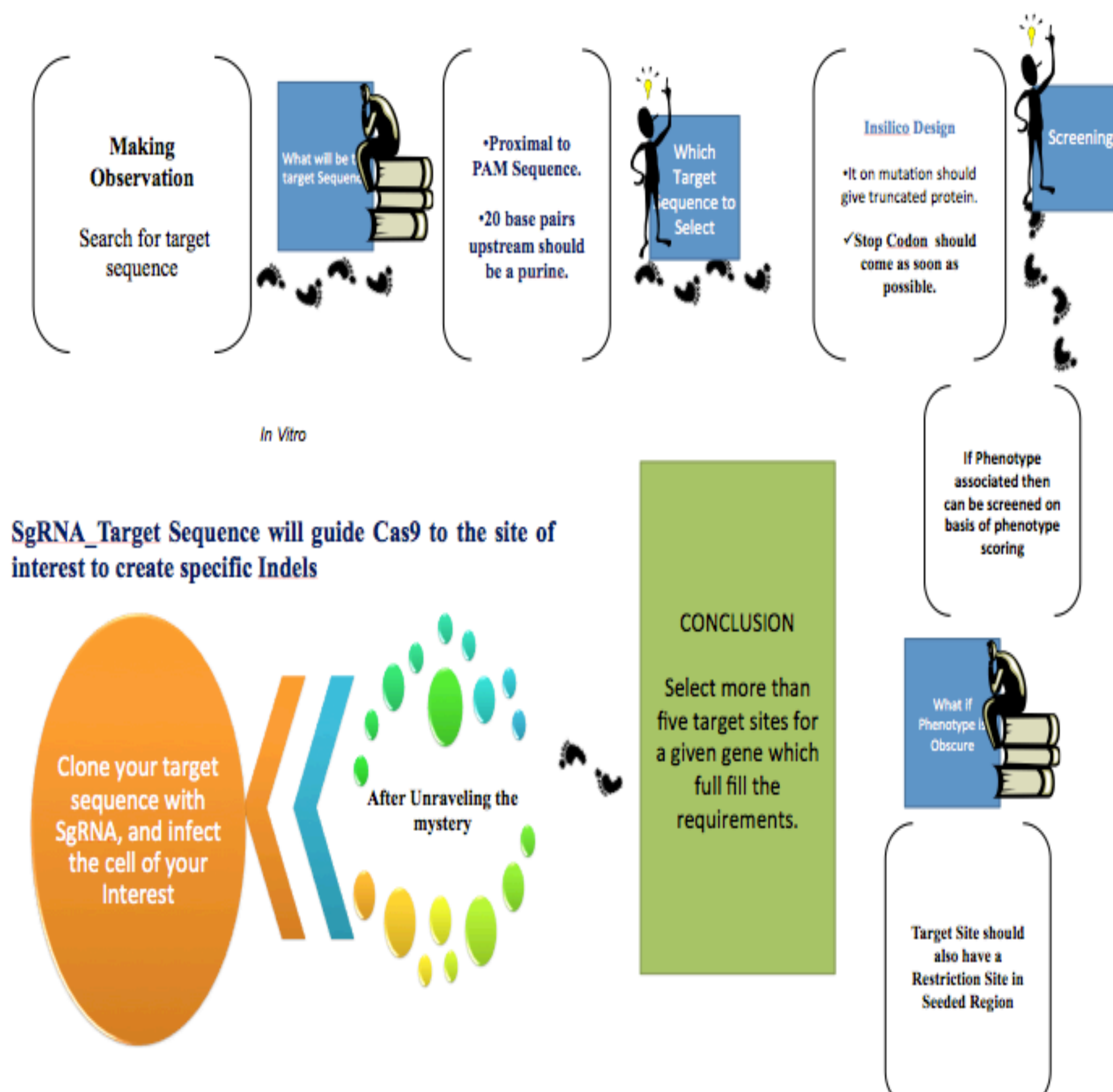


Figure 2: Most efficient Genetic Engineering tool

Strategy



Cloning Target vector.

1. Peft-3_Cas-9-SV40-NLS_tbb-2 3'UTR
2. pU6_CONTROL_SgRNA
3. pU6-BseR1-BseR1-SgRNA

pU6-BseRI-sgRNA

pU6 -G(inserted before N20)-BseRI- BseRI----- U6 3'UTR
TAAATGTTTGAATTTTCTCTCCTCactttcGAGGAGATTAAAGAGTTTTAG
AGCT
ATTACAAACTTAAAGAGAGGAGtgaaagCTCCTCTAATTTCTCAAAT
CTCGA

After BseRI digestion

TAAATGTTTG-----TTTAGAGCT
ATTACAA-----CAAATCTCGA

Primers to be annealed and inserted

Primer 1 (forward): (N20)GT
Primer 2 (reverse): AC(N20)

Results



Discussion

The research project can open a novel window in the field of regeneration, and can lead to develop the methodology to enhance regenerative capacity in animal models, which have lost it.

Acknowledgments

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