Studying the Mechanism of CRISPR-Cas Acquisition in *S. pyogenes*

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Abstract

- In bacteria and archaea, clustered regularly interspaced short palindromic repeat (CRISPR) provide adaptive immunity against viral infection.
- Universally conserved CRISPR associated (Cas) proteins, such as Cas1 and Cas2, have the ability to acquire viral DNA into the CRISPR locus as a new spacer.
- In this study, we have tried to develop a screen for studying the site, sequences and orientation of the integrated protospacers in *S. pyogenes*.
- By developing such a screen, it would create a platform to fully understand CRISPR's evolution and further target specificity of crRNA-effector complexes.

Introduction

Fig 1. Overview of CRISPR-Cas System

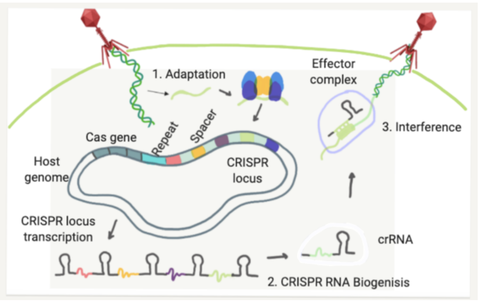


Fig 2. Architecture of the Cas1 and Cas2 proteins. Their structure comprises four copies of Cas1 and two copies of Cas2. Cas1 proteins ar the integrases and Cas2 is a structural protein for maintain the proper orientation for protospacer integration.

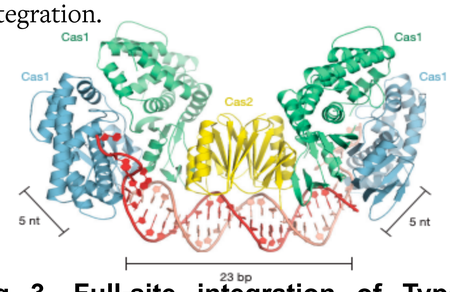
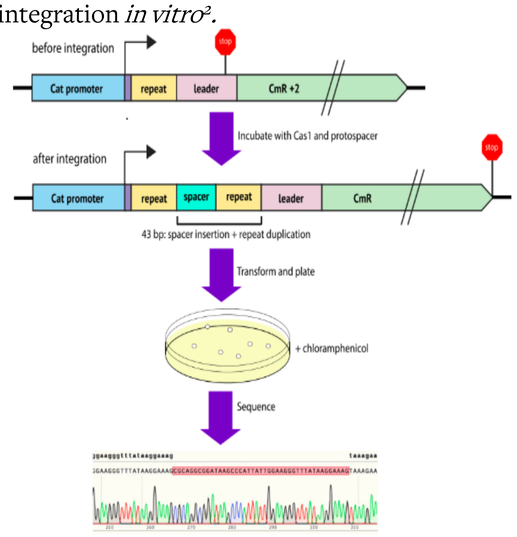


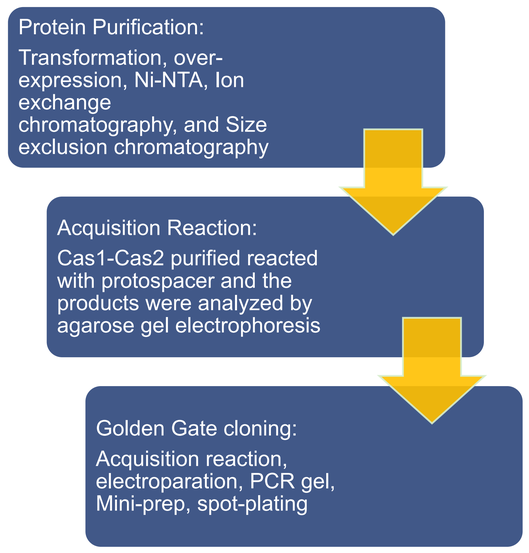
Fig 3. Full-site integration of Type V systems:. A study designed a screen for protospacer integration in which they found that V-C Cas1 has the ability to carry out full-site integration *in vitro*[2].



Objective

In this study, we aim to develop a similar selection screen for protospacers that efficiently undergo full-site integration in *Streptococcus pyogenes.*

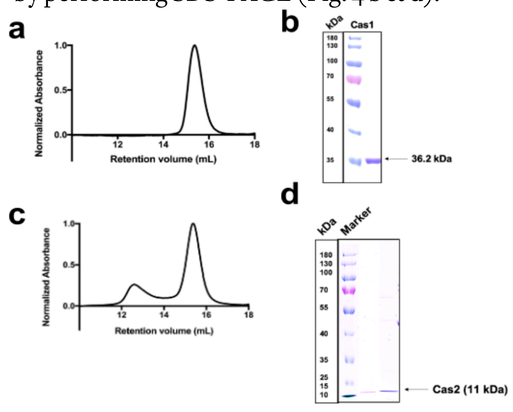
Methodology



Results

Figure 4 | Purification of Cas1 and Cas2.

- The purification profile of size-exclusion chromatography for the Cas1 and Cas2 proteins (Fig. 4 a & b).
- The purity of the purified products was assessed by performing SDS-PAGE (Fig. 4 b & d).



Results

Figure 5 | Chloramphenicol plasmid integrated to pCRISPR.

- The pCRISPR containing an Ampicillin resistance gene and the donor vector for the chloramphenicol resistance gene has been depicted in Figure 2 (a) and (b) respectively.

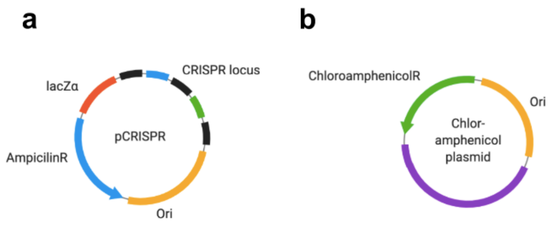
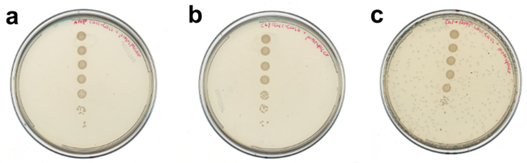


Figure 6 | Electroporation transformation.

- Colonies obtained on plates containing (a) ampicillin, (b) chloramphenicol, and (c) ampicillin + chloramphenicol



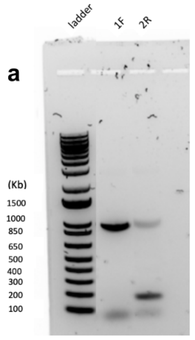


Figure 7 | Chloramphenicol was successfully integrated in pCRISPR

- We performed PCR reactions with two different sets of primers that would identify the orientation of the reaction products.

Results

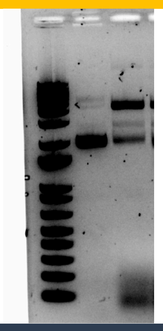


Figure 8 | Cas 1 and Cas 2 protospacer integration

- Cas1 and Cas2 cause the nicking of the supercoiled plasmid which then migrates slowly on the agarose gel as compared to the supercoiled plasmid. Hence, the indicative of integration

Conclusion

- We were able to successfully develop a screen protospacer integration in Type-II A system in *Streptococcus pyogenes.*
- It is effective to test protospacer integration with purified Cas1 and Cas2 proteins
- It appears to be that using the chloramphenicol cassette with Golden Gate compatible sites is successful in testing the site, orientation, and sequence of protospacer integration.

Future Work

- However, the screen needs to be validated further with methods such as Sanger sequencing and restriction digestion
- Next-generation sequencing can also be performed on the reaction products to study a population

References

[1] Nuñez, James K., *et al.* Foreign DNA Capture during CRISPR–Cas Adaptive Immunity. *Nature*, **527**, 535–538. (2015) doi:10.1038/nature15760.
[2] Nuñez, James K., *et al.* Integrase-Mediated Spacer Acquisition during CRISPR–Cas Adaptive Immunity. *Nature*, **519**, 193–198. (2015) doi:10.1038/nature14237.

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