The efficiency of genome editing by a small Cas9 orthologue derived from Campylobacter jejuni (CjeCas9)

Yumiko Kimura1, Ciaran Lee2, Harshavardhan Deshmukh2, Anirban Ray2, Tim Davis2 and Gang Bao2

1 Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Chiba, Japan 2 Department of Bioengineering, Rice University, Houston, TX, U.S.

Introduction

What is ‘Genome editing’?
- A type of genetic engineering in which DNA is inserted, deleted or replaced in the genome of a living organism using engineered nucleases, or “molecular scissors”.

How is it used?
- Stem cell engineering & Gene therapy for diseases such as hemoglobinopathies (SCD and β-thalassemia), HIV, Huntington’s disease, cancer.
- Disease-resistant transgenic plants
- Tissue & Animal disease models

About CRISPR/Cas9
- Discovered as the immune system in bacteria and other group
- The CRISPR-Cas system is a revolutionary technique because it is a fast, cheap, efficient method for editing DNA.
- Applied in a diverse set of species
- Potential for off-target effects. It becomes a problem when it used in vivo.
- Complex of guide RNA and Cas9

Motivation

Different species of bacteria have different Cas9 orthologues and they recognize different protospacer adjacent motifs (PAMs).
- Cas9 orthologue derived from Streptococcus pyogenes → SpCas9
  168bp 5’-NGG-3’ PAM
- Cas9 orthologue derived from Staphylococcus aureus → SaCas9
  105bp 5’-INNGRRRT-3’ PAM
- Cas9 orthologue derived from Campylobacter jejuni → CjeCas9
  98bp

During this research project, I surveyed the efficiency of genome editing by CjeCas9. I assayed
- The ability of the CjeCas9 nuclease to recognize different PAMs
- The efficiency of DNA targeting with different guide RNA (gRNA) lengths

Method

Step 1:
- The DNA oligos were phosphorylated and sense and anti-sense oligos were annealed prior to cloning into the expression vector.
- To facilitate cloning of gRNA sequences downstream of the RNA pol III promoter U6, the CjeCas9 expression plasmid was digested using the restriction enzyme BbsI. The annealed oligos were cloned into the digested plasmid using T4 DNA ligase IV

Step 2:
- We picked up 2 colonies from each plate and inoculated into 3 mL of LB culture medium supplemented with ampicillin and cultured overnight.

Step 3:
- CjeCas9 plasmids were extracted from E.coli and sent for sequencing to confirm the presence of the gRNA sequence.

Step 4:
- CjeCas9 plasmids were chemically transfected into HEK293-GFP cells.

Step 5:
- We measured GFP expression by using Flow cytometry.
- The level of GFP knock-out achieved reflects the efficiency of genome editing by CjeCas9.

Result

Conclusion

Though we measured GFP levels until Day8, we could not see any drop of GFP expression. In Step 4, we confirmed the presence of the gRNA sequence, so it appears that the CjeCas9 is not a particularly active orthologue. To confirm these findings, other members of the Bao Lab will try Step 4, 5, and 6 of the procedure again.

In this project we did not observe effective DNA cutting with CjeCas9. However, CjeCas9 is smaller than SpCas9 and SaCas9 which is advantageous for in vivo delivery applications. Also, it may have high specificity due to the requirement for a longer PAM sequence although its efficiency of DNA targeting with different guide RNA (gRNA) lengths

Reference


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