Introduction

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated endonucleases (Cas) were first found to be involved in microbial adaptive immunity. CRISPR and Cas9 are now used for gene editing.

Gene Editing Steps
1. guide RNA (gRNA) is designed to contain a targeting guide sequence.
2. gRNA forms a complex with Cas9 and directs Cas9 to the target site.
3. Cas9 cleaves the target site and makes a double strand break.
4. Error prone nonhomologous end joining (NHEJ) happens with insert or deletion.
5. Homologous recombination (HR) happens when donor DNA with homologous sequence is present.

Mechanism of gene targeting
Figure 1. Mechanism of gene targeting using CRISPR/Cas9 (adapted from [2])

Figure 2. Gene Repair mechanism

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Single Strand Annealing (SSA) assay
Quantify the activity of Cas9/gRNA by luminescence.

Figure 3. Mechanism of SSA assay

Methodology

Overview of experiments

1. Cloning
   15 disease causing targets were selected. When mutated these genes result in cystic fibrosis, tyrosinemia and SCID. These target sites were cloned into the SSA vector.

2. Transformation
   Ligated plasmids were inserted into chemically competent E.coli.

3. Cell culture
   Cells were grown O/N, colonies picked and grown O/N in liquid media.

4. Colony PCR, Miniprep, Sequencing
   Clones screened by colony PCR. Correct clones were confirmed by Sanger Sequencing.

5. Triple Transfection
   HEK293T cells were co-transfected with gRNA/saCas9 dual expressing plasmid and Renilla plasmid.

6. SSA assay
   Cleavage of the target resulted in luminescence.

Results

SSA assay
2 target sequences were correctly inserted inside the SSA target vector. The targets were for genes that result in hemophilia A (F8) and cystic fibrosis (CFTR) when mutated.

Motivation

- Determine whether SSA assay can be used to assess gRNA/Cas9 activity compared with T7E1 assay.
- Compare the cleavage activity of Cas9/gRNA at different target sites.

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References

[1] Patrick D. Hsu, Cell (2014)