1 Introduction

A mechanistic model of CRISPR/Cas9 gene isertion is presented.

1.1 System of reaction diagrams

Lets denote our plasmid $CRISPeasy$, then the replication rate of our vector is expressed as

$$\xrightarrow{k} CRISPeasy \xrightarrow{\delta}$$

where k is the vector replication rate and δ its degradation rate. The degradation of Arabinose and IPTG as well as the production of Cas9 protein controled by Arabinose, Recombinase A protein controled by IPTG and sgRNA is expressed as

$$Arabinose \xrightarrow{\delta_{Arabinose}}$$
$$IPTG \xrightarrow{\delta_{IPTG}}$$
$$Cas9' + Arabinose \xrightarrow{k_{Cas9}} Cas9 \xrightarrow{\delta_{Cas9}}$$
$$RecA' + IPTG \xrightarrow{k_{RecA}} RecA \xrightarrow{\delta_{RecA}}$$
$$sgRNA' \xrightarrow{k_{sgRNA}} sgRNA \xrightarrow{\delta_{sgRNA}}$$

where $Cas9'$, $RecA'$ and $sgRNA'$ are DNA concentration of the respective proteins, k_{Cas9}, k_{RecA} are the translation plus the transcription rates and k_{sgRNA} is the transcription rate. Also, we denote $\delta_{Arabinose}$, δ_{IPTG}, δ_{Cas9}, δ_{RecA} and δ_{sgRNA} as the rates of degradation of each one of the organisms.

The protein $Cas9$ binds with $sgRNA$ to form the complex C_1.

$$Cas9 + sgRNA \xrightarrow{k_1} C_1$$

The complex C_1 diffuses to the target site for the cleavage. Denote the complex formed by C_1 with the $gDNA$ as C_2 , and $gDNA^*$ as the cleaved $gDNA$.

$$C_1 + gDNA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_2 \xrightarrow{k_*} gDNA^*$$

Denote $gDNA^{**}$ as the $gDNA$ cleaved and partially digested forming sticky ends due to RecBCD. Where C_3 is the intermediate complex formed by RecBCD and $gDNA^*$.

$$gDNA^* + RecBCD \xrightarrow{k_3} C_3 \xrightarrow{k_{**}} gDNA^{**} + RecBCD$$

Recombinase A protein joins the sticky ends of $gDNA$ to form the complex C_4.

$$gDNA^{**} + RecA \xrightarrow{k_4} C_4$$

The complex C_4 joins the $CRISPeasy$ vector to form the complex C_5 and seeks for homology. When homology is found, DNA Polymerase joins C_5 and

fills the empty space. We will note $gDNA_{\#}$ as the genomic DNA repaired with the *rfp* gene inserted.

$$C_4 + CRISPeasy \xrightarrow{k_5} C_5 \xrightarrow{k_{\#}} gDNA_{\#} + RecA + CRISP_{easy}$$

1.2 Differential equations

We want to model the variation of modified genomic DNA, ie. with *rfp* gene inserted, which we denoted as $gDNA_{\#}$. Using the Law of mass action we can deduce from the reaction diagrams the following systems of differential equations govering the system.

- $\frac{d[Cas9]}{dt} = k_{Cas9}[Cas9'][Arabinose] - \delta_{Cas9}[Cas9] - k_1[Cas9][sgRNA]$
- $\frac{d[Arabinose]}{dt} = -\delta_{Arabinose}[Arabinose] - k_{Cas9}[Cas9'][Arabinose]$
- $\frac{d[sgRNA]}{dt} = k_{sgRNA}[sgRNA'] - \delta_{sgRNA}[sgRNA] - k_1[Cas9][sgRNA]$
- $\frac{d[RecA]}{dt} = k_{RecA}[RecA'][IPTG] - \delta_{RecA}[RecA] - k_4[RecA][gDNA^{**}] + k_{\#}[C_5]$
- $\frac{d[IPTG]}{dt} = -k_{RecA}[IPTG][RecA'] - \delta_{IPTG}[IPTG]$
- $\frac{d[C_1]}{dt} = k_1[Cas9][sgRNA] + k_{-2}[C_2] - k_2[C_1][gDNA]$
- $\frac{d[C_2]}{dt} = k_2[C_1][gDNA] - k_{-2}[C_2] - k_*[C_2]$
- $\frac{d[gDNA^*]}{dt} = k_*[C_2] - k_3[gDNA^*][RecBCD]$
- $\frac{d[C_3]}{dt} = k_3[gDNA^*][RecBCD] - k_{**}[C_3]$
- $\frac{d[gDNA^{**}]}{dt} = k_{**}[C_3] - k_4[gDNA^{**}][RecA]$
- $\frac{d[C_4]}{dt} = k_4[gDNA^{**}][RecA] - k_5[C_4][CRISPeasy]$
- $\frac{d[C_5]}{dt} = k_5[C_4][CRISPeasy] - k_{\#}[C_5]$
- $\frac{d[gDNA_{\#}]}{dt} = k_{\#}[C_5]$