

1 Introduction

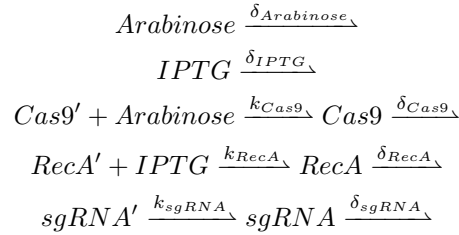
A mechanistic model of CRISPR/Cas9 gene insertion is presented.

1.1 System of reaction diagrams

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



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 controlled by Arabinose, Recombinase A protein controlled by IPTG and sgRNA is expressed as

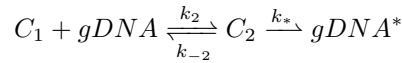


where $Cas9'$, $RecA'$ and $sgRNA'$ are DNA concentration of the respective proteins, k_{Cas9} , k_{RecA} are the translation plus the transcription rates and k_{sgRNA} is the transcription rate. Also, we denote $\delta_{Arabinose}$, δ_{IPTG} , δ_{Cas9} , δ_{RecA} and δ_{sgRNA} as the rates of degradation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

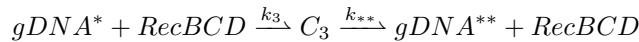
The protein $Cas9$ binds with $sgRNA$ to form the complex C_1 .



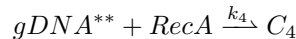
The complex C_1 diffuses to the target site for the cleavage. Denote the complex formed by C_1 with the $gDNA$ as C_2 , and $gDNA^*$ as the cleaved $gDNA$.



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 $gDNA^*$.

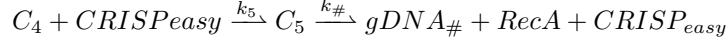


Recombinase A protein joins the sticky ends of $gDNA$ to form the complex C_4 .



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.



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 governing the system.

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