MATHEMATICAL MODELING OF RECOMBINANT DNA EXPRESSION

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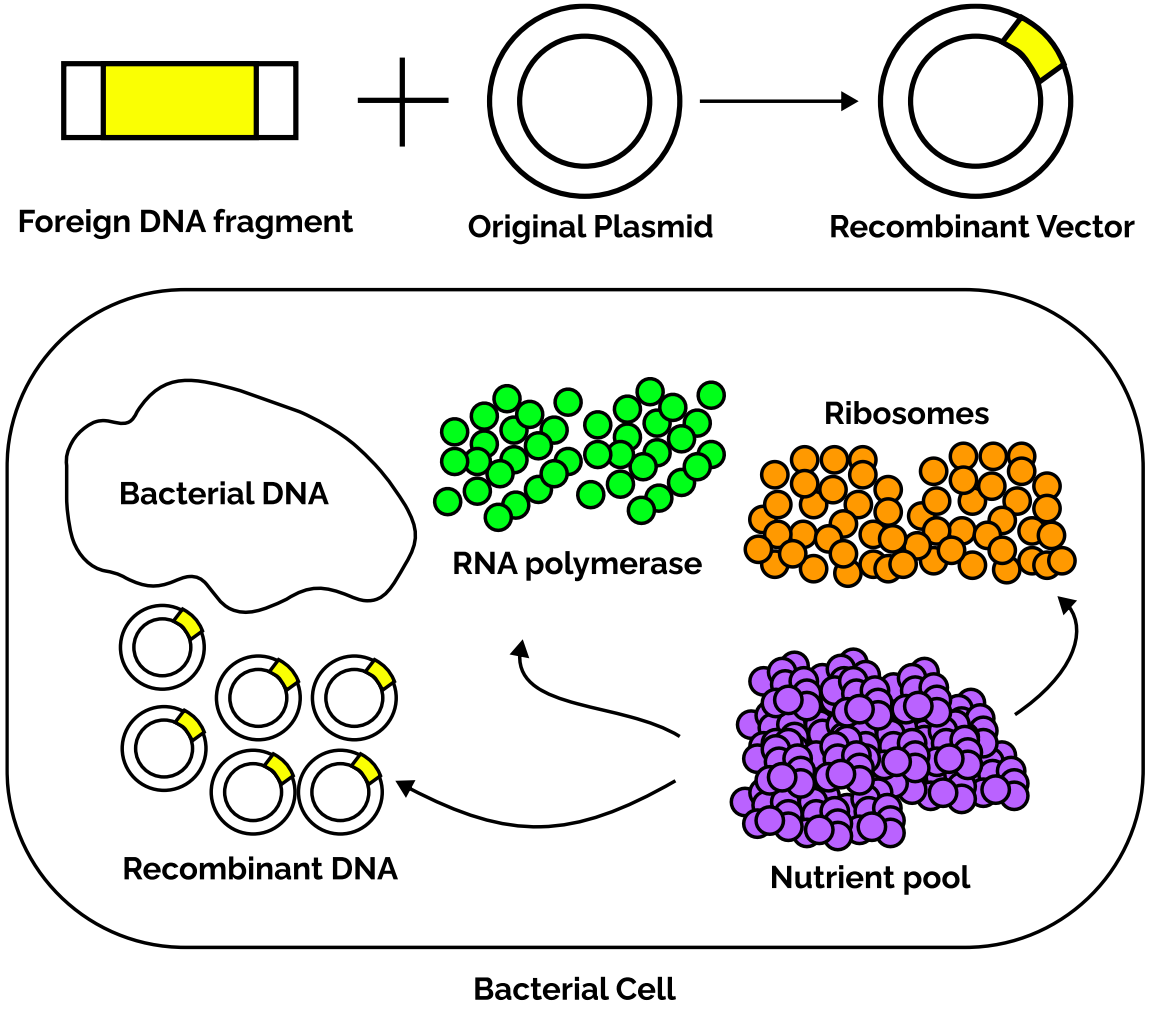
INTRODUCTION

INTRODUCTION TO DNA RECOMBINATION

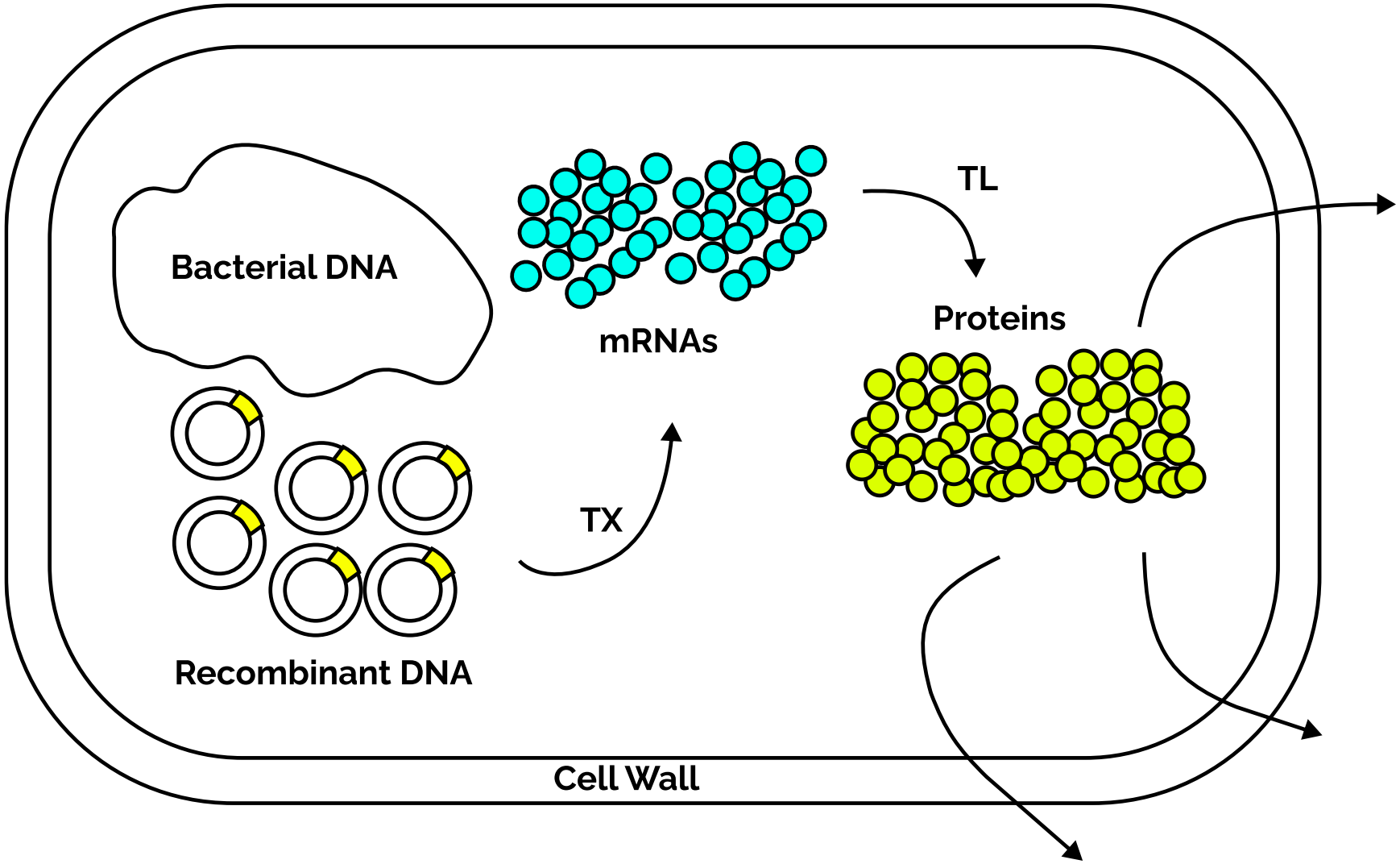
Genetic Engineering

- Most proteins are difficult to manufacture with available synthetic capabilities
- Genes that are involved in producing protein molecules can be assembled through *gene manipulation* to a *cistron*
- The cistron encodes necessary information and utilize intrinsic gene expression machinery to produce target proteins



INTRODUCTION TO DNA RECOMBINATION

Gene expression



OBJECTIVES

General Objective

- To develop a minimalistic mathematical model that grasps the basic mechanics of recombinant DNA expression

Specific Objectives

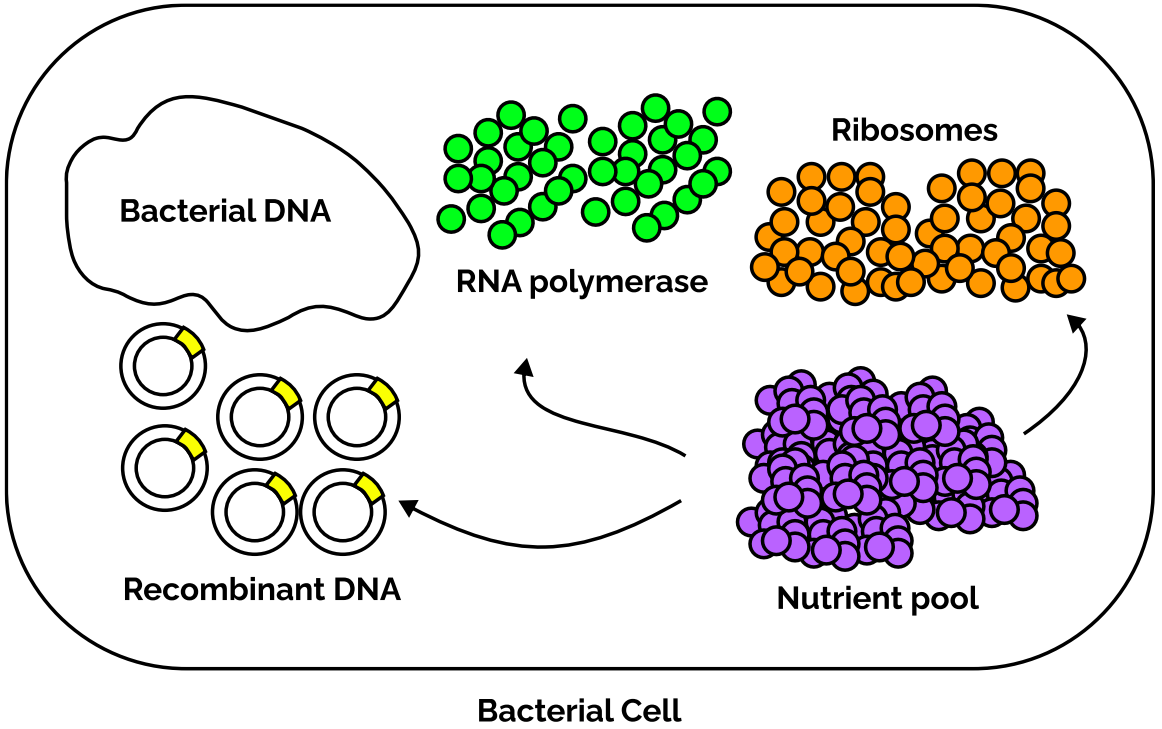
- To understand the crucial checkpoints of protein expression
- To understand the relationship between host history and protein production

MODEL DEVELOPMENT

MODEL DEVELOPMENT

STEP 1
Assumptions

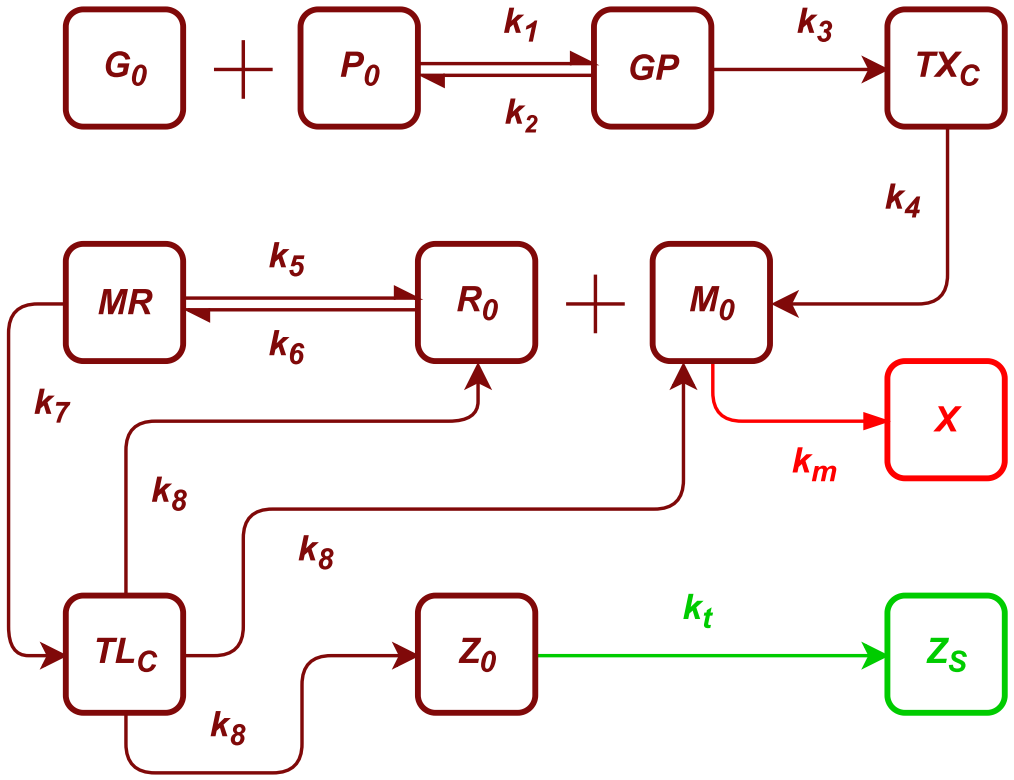
1. The bacterial cytoplasm is assumed to be homogenous and posses a fixed volume
2. The biochemical nutrient pool is large and the changes during consumption is negligible
3. No replication occurs within the simulation time/observation time
4. The resources available for expression is assumed to be in high numbers and continuum hypothesis and mass action-based formalisms become applicable
5. The synthesized proteins are assumed to be transported outside (i.e., to culture supernatant)



MODEL DEVELOPMENT

STEP 2

Understanding the biochemical interactions and developing a chemical reaction network



G_0 – plasmid

P_0 – RNA polymerase (RNAP)

GP – RNAP-plasmid complex

TX_c – escape committed complex

M_0 – mRNA

R_0 – ribosome

MR – mRNA-ribosome complex

TL_c – translation complex

Z_0 – reporter protein

Z_s – secreted protein

k1 – RNAP-promoter association

k2 – RNAP-promoter dissociation

k3 – transcription commitment

k4 – mRNA production

k5 – mRNA-ribosome association

k6 – mRNA-ribosome dissociation

k7 – translation commitment

k8 – protein production

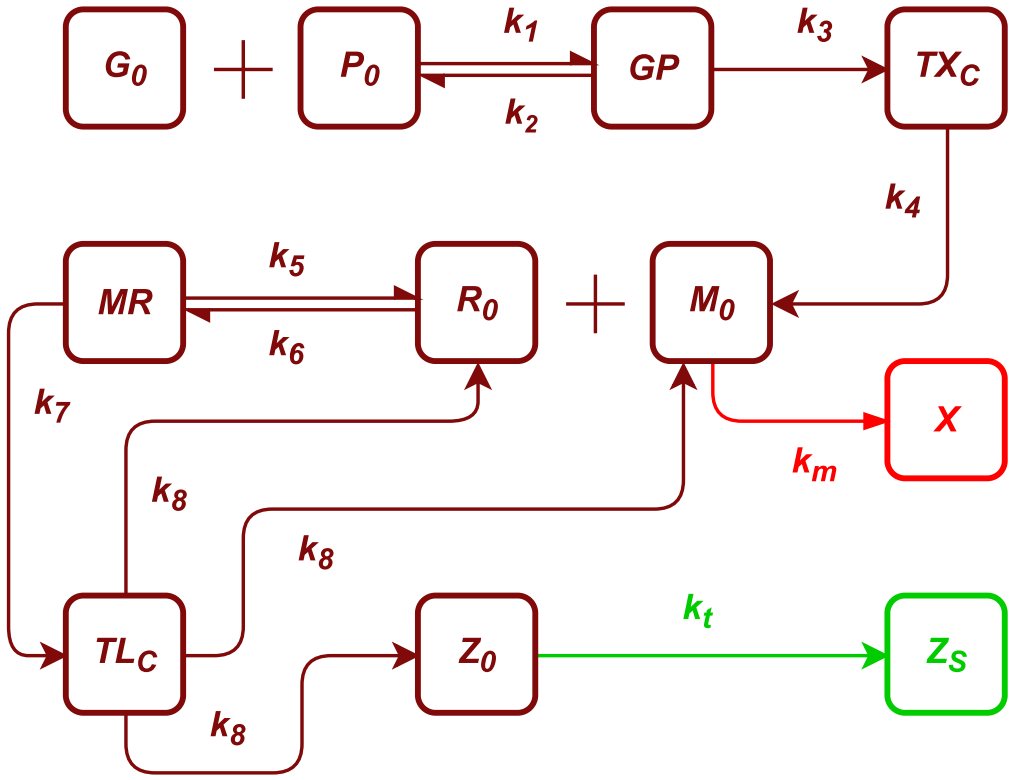
k_m – mRNA degradation

k_t – protein transport

MODEL DEVELOPMENT

STEP 3

Develop differential equations for the formulated reaction network



Differential Equations
$dG_0 = -k_1G_0P_0 + k_2GP + k_4TX_C$
$dP_0 = -k_1G_0P_0 + k_2GP + k_4TX_C$
$dGP = k_1G_0P_0 - k_2GP - k_3GP$
$dTX_C = k3GP - k4TX_C$
$dM_0 = k_4TX_C - k_mM_0 - k_5M_0R_0 + k_6MR + k_8TL_C$
$dR_0 = -k_5M_0R_0 + k_6MR + k_8TL_C$
$dMR = k_5M_0R_0 - k_6MR - k_7MR$
$dTL_C = k_7MR - k_8TL_C$
$dZ_0 = k_8TL_C - k_tZ_0$
$dZ_S = k_tZ_0$

MODEL DEVELOPMENT

STEP 4

Carry out simulation for a collection of time points using a computational software (*R* and the package *deSolve*)

Initial Concentrations	
G0 – 0.5 nM	TL_C – 0 nM
P0 – 0.5 nM	Z0 – 0 nM
GP – 0 nM	Zs – 0 nM
TX_C – 0 nM	M0 – 0 nM
R0 – 0.5 nM	MR – 0 nM

Rate Constants	
k_1 – $6x10^9$ $M^{-1}min^{-1}$	k_7 – 30 min^{-1}
k_2 – $6x10^9$ $M^{-1}min^{-1}$	k_8 – 0.9 min^{-1}
k_3 – 60 min^{-1}	k_t – 60 min^{-1}
k_4 – 0.9 min^{-1}	k_m – 18 min^{-1}
k_5 – $6x10^9$ $M^{-1}min^{-1}$	k_6 – 135 min^{-1}

Code Availability

https://github.com/zachariah-ibrahim/recombinant-DNA-expression

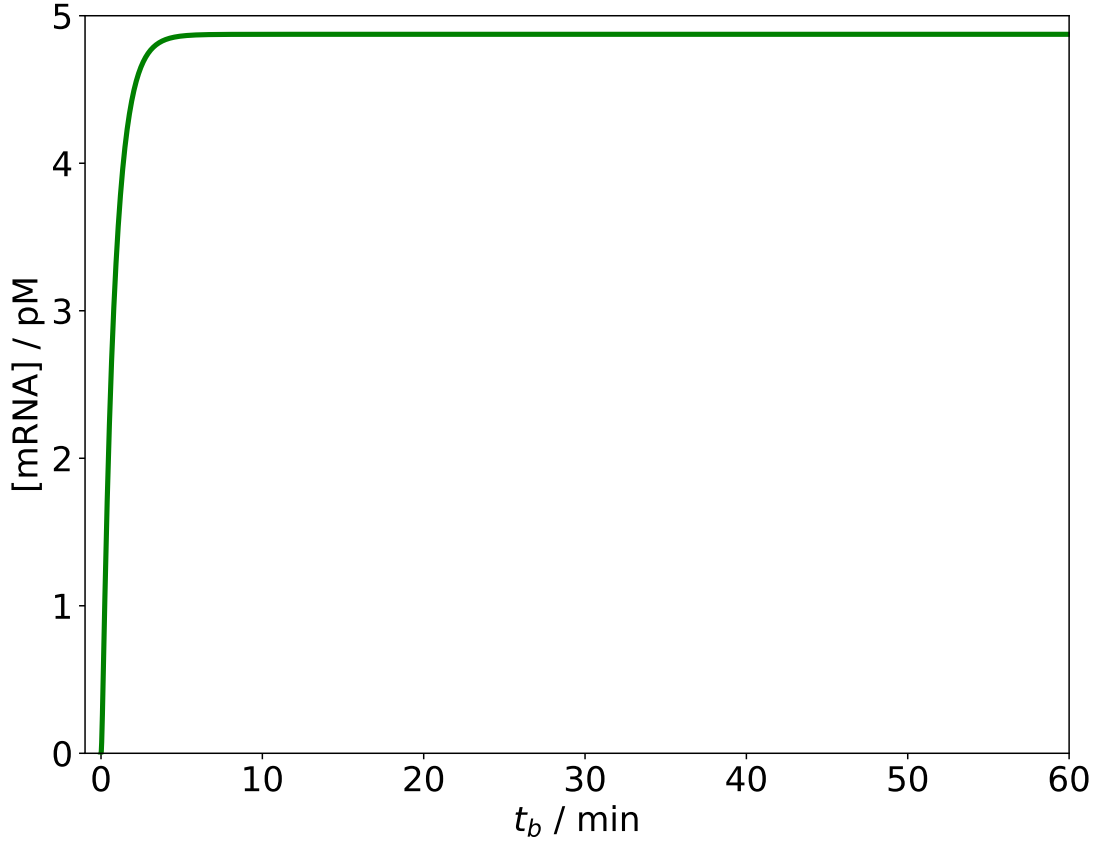
Parameter availability

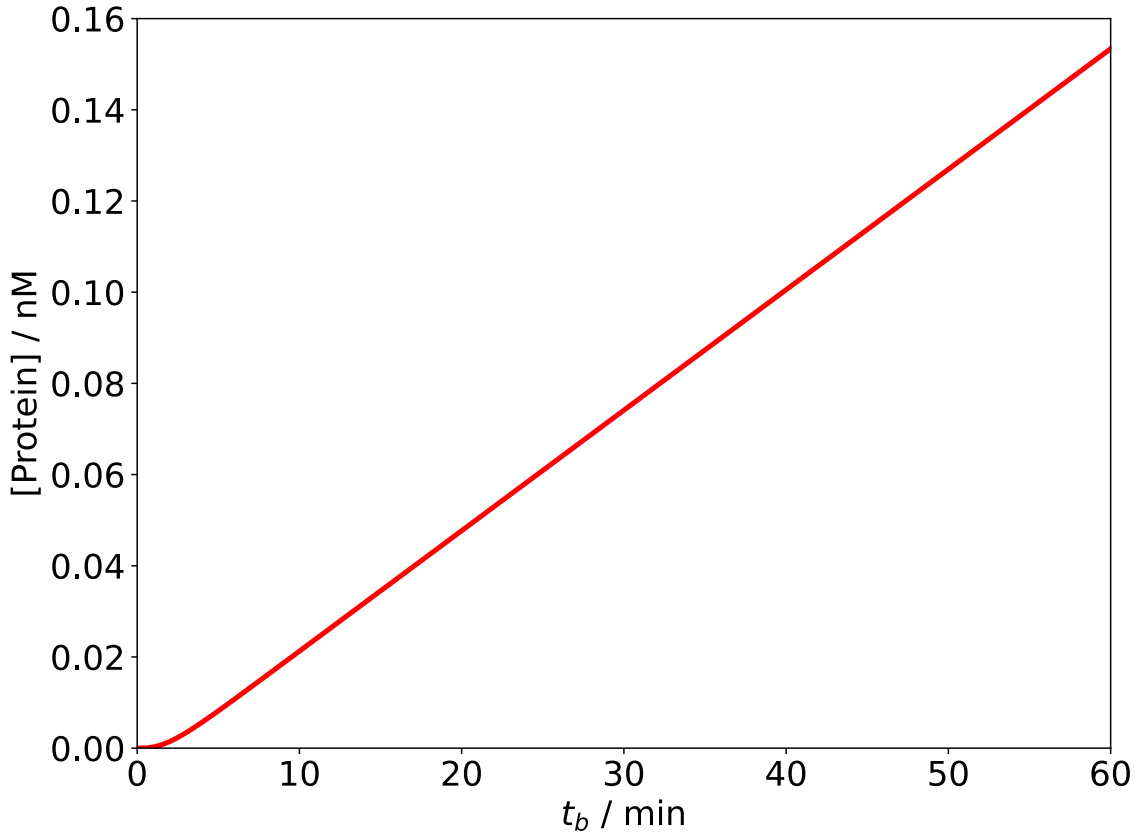
Kierzek, A. M., Zaim, J., & Zielenkiewicz, P. (2001). The effect of transcription and translation initiation frequencies on the stochastic fluctuations in prokaryotic gene expression. The Journal of Biological Chemistry, 276(11), 8165–8172. doi:10.1074/jbc.M006264200

RESULTS & DISCUSSION

RESULTS & DISCUSSION

Simulation results (Default Settings)

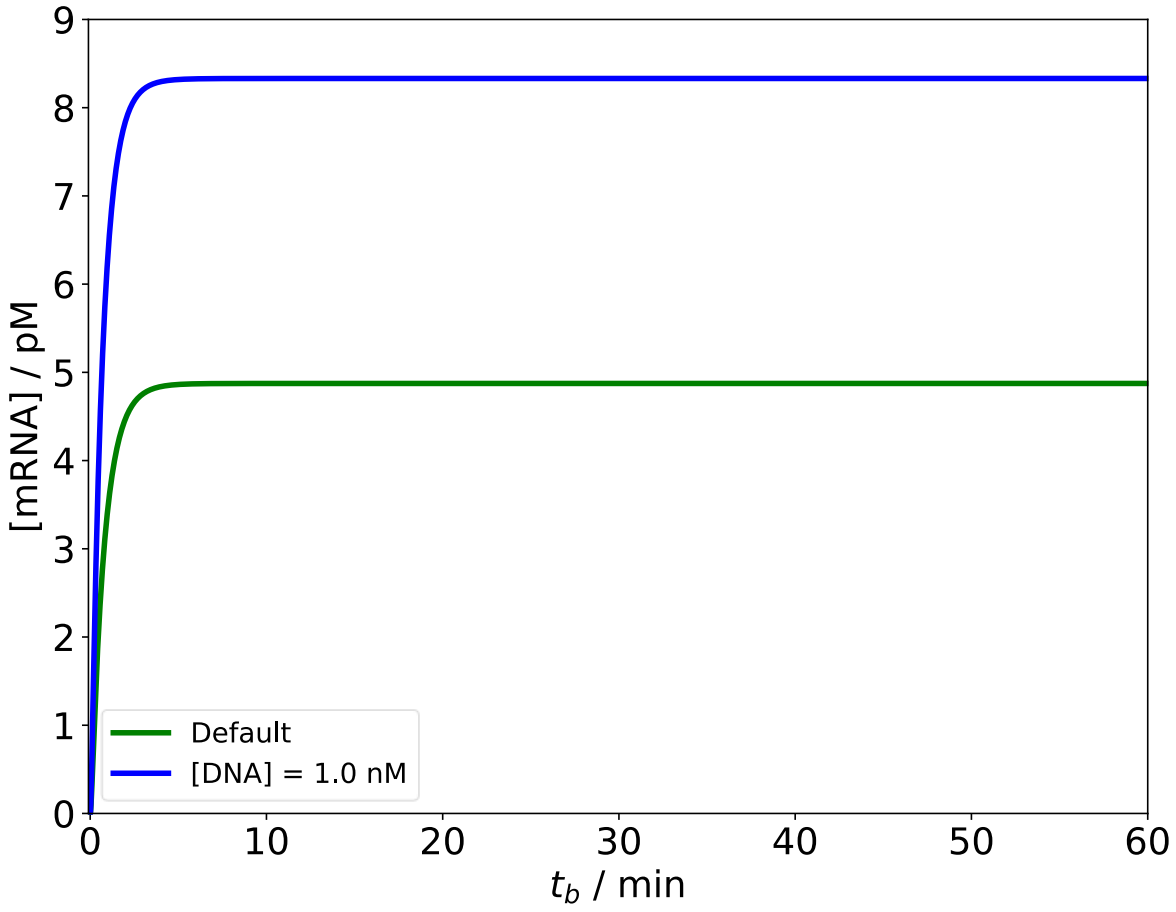


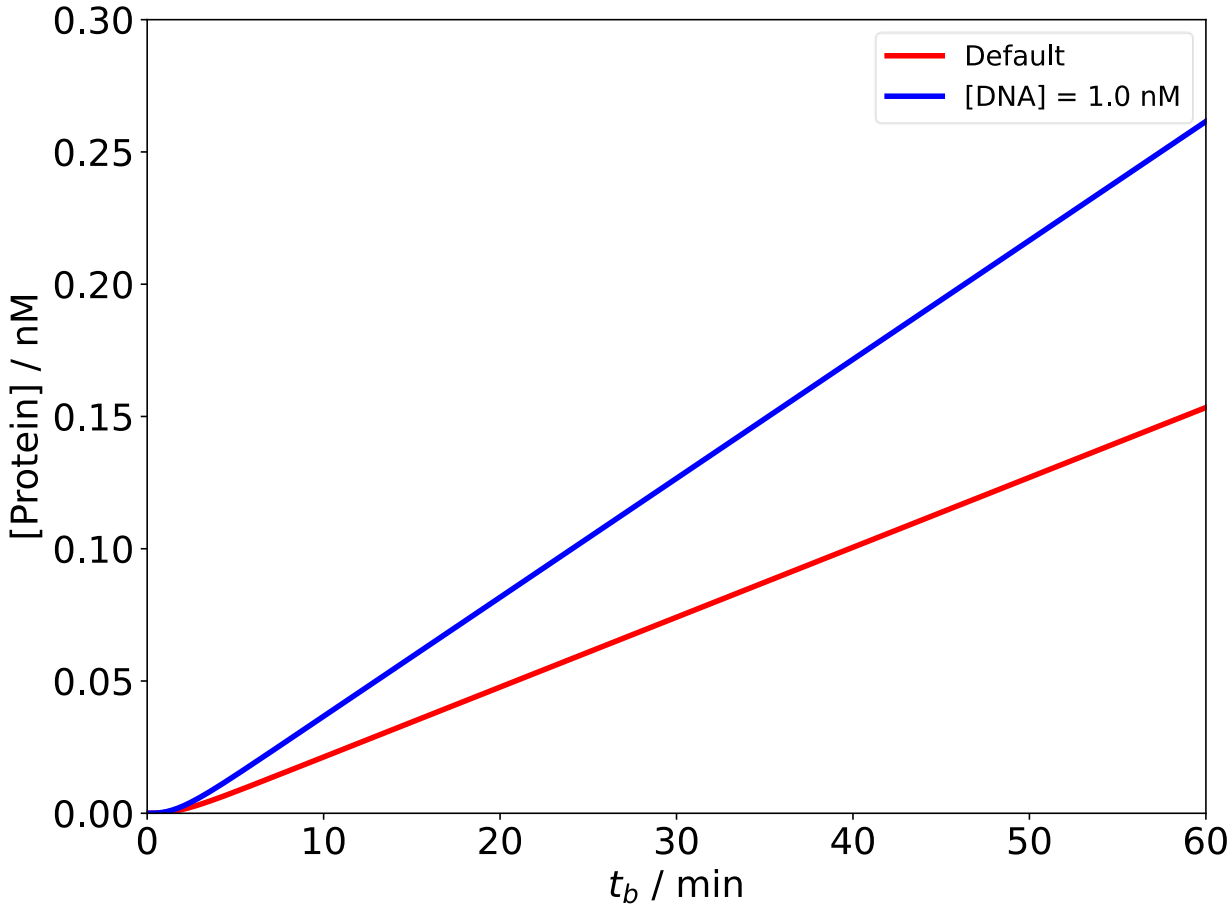


- **mRNA within the bacterial cell shows saturation behavior**
- **The protein production show a quasi-linear behavior**

RESULTS & DISCUSSION

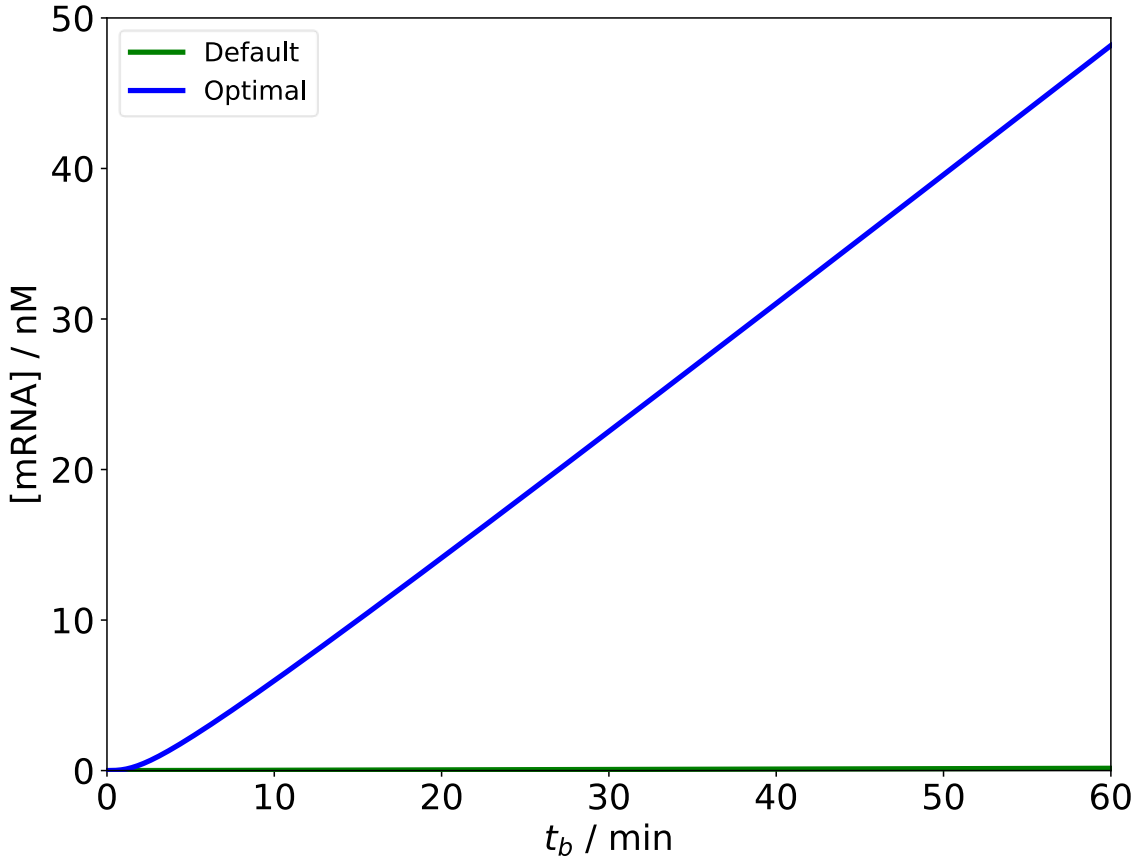
Simulation results (High copy number)

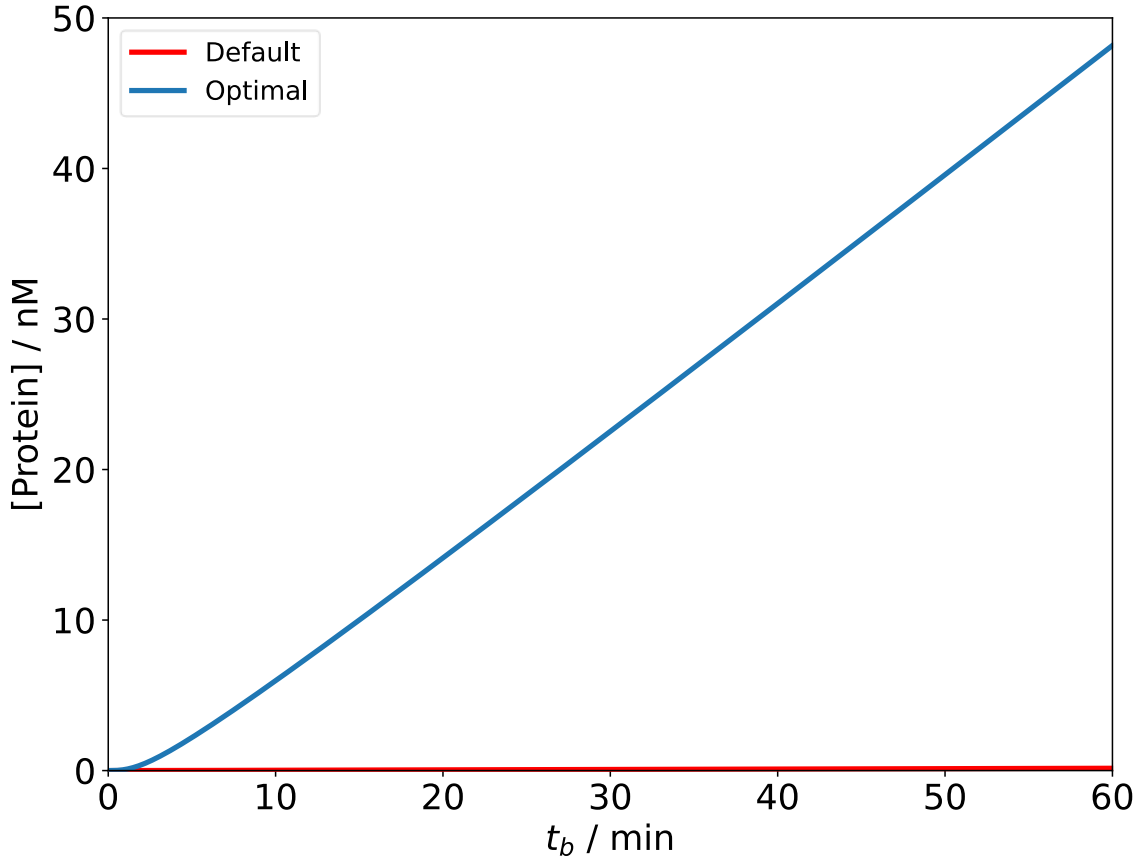




RESULTS & DISCUSSION

Simulation results (High copy number + no mRNA degradation + increased nutrient pool)





CONCLUSION

- **Positive modifications can improve the production yield significantly**
- **Choosing a better candidate/host is also a promising approach to improve yield**

THANK YOU