CHAPTER 3: RESULTS

3.1 Dynamics of C2-FFL with AND/OR gate

Figure 3.1 shows the dynamics for C2-FFL with AND gate. At the ON step, transcription factor X is activated by its inducer, causing the concentration of its active form X^* to increase. Protein Y and Z begin to decrease when X^* exceeds K_{xy} and K_{xz}. At the OFF step, the activation signal for protein X is removed. When X^* falls below K_{xy} and K_{xz}, the production of protein Y resumes. In C2-FFL with AND gate, the production of protein Z requires the concentration of active form of protein Y exceeding K_{yz} and the concentration of active form of X falling below $K_{xz.}$ Only when this condition is achieved will the production of protein Z begin. This explains the delay in activation of protein Z production. The delay lengthens the response time (time taken to reach half of the steady-state concentration) of C2-FFL.

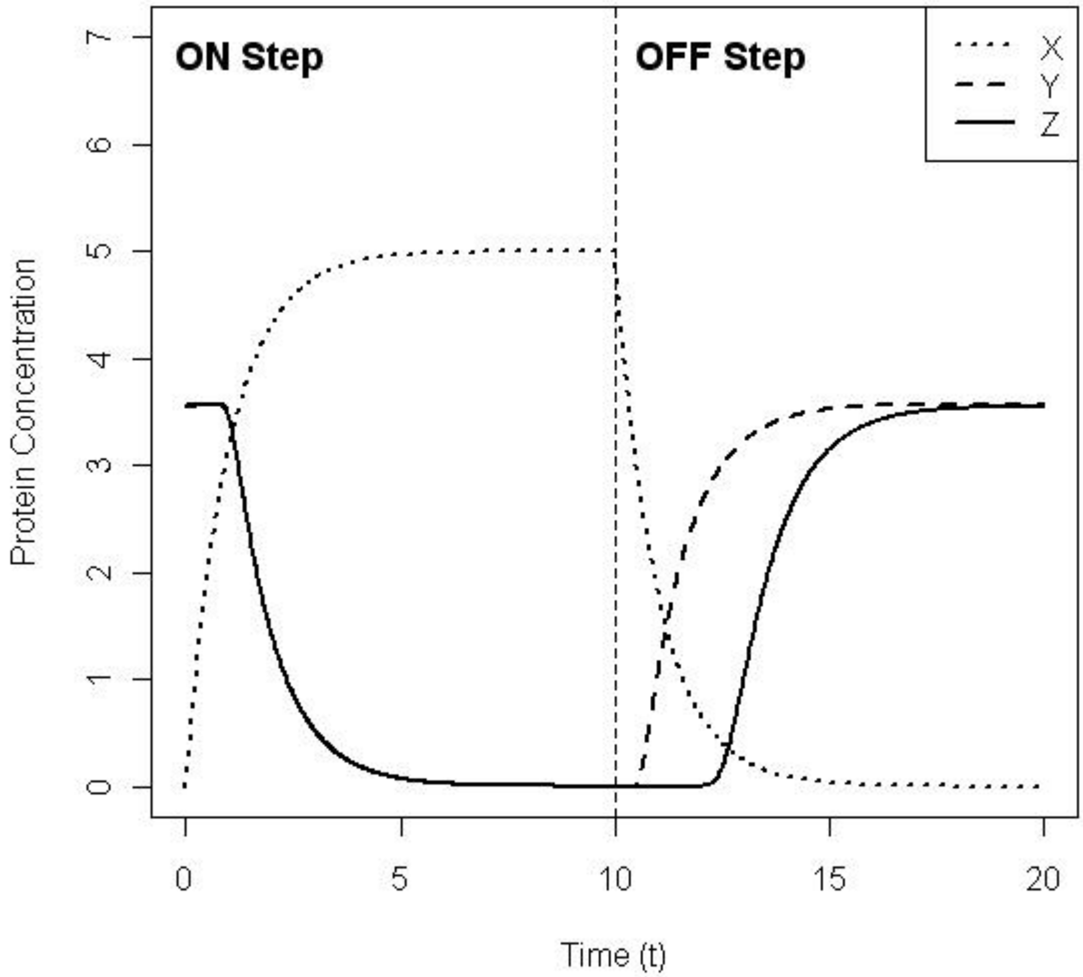

Figure 3.1: Dynamics of C2-FFL with AND gate at ON and OFF step.

In C2-FFL with OR gate (Figure 3.2) at the ON step, protein *Z* will be produced as long as the active form of *Y* exceeds K_{yz}. When protein *X* is activated by its inducer and the concentration of its active form X^* reaches K_{xy} and K_{xz}, the production of protein *Y* is repressed. The concentration of protein *Z* remains unaffected for a while because the amount of *Y* is still sufficient to maintain its production. When the concentration of protein *Y* falls below K_{yz}, the production of protein *Z* ceases, causing its concentration to decrease.

Thus, there is a short delay in repression of protein *Z* production. At the OFF step, once the concentration of X^* falls below K_{xz} and K_{xy}, it is not able to repress the production of protein *Y* and *Z*, and basically the network degenerates into simple regulation of Z by *Y*. There is only a short delay in the production of protein *Z*, with corresponding shorter response time.

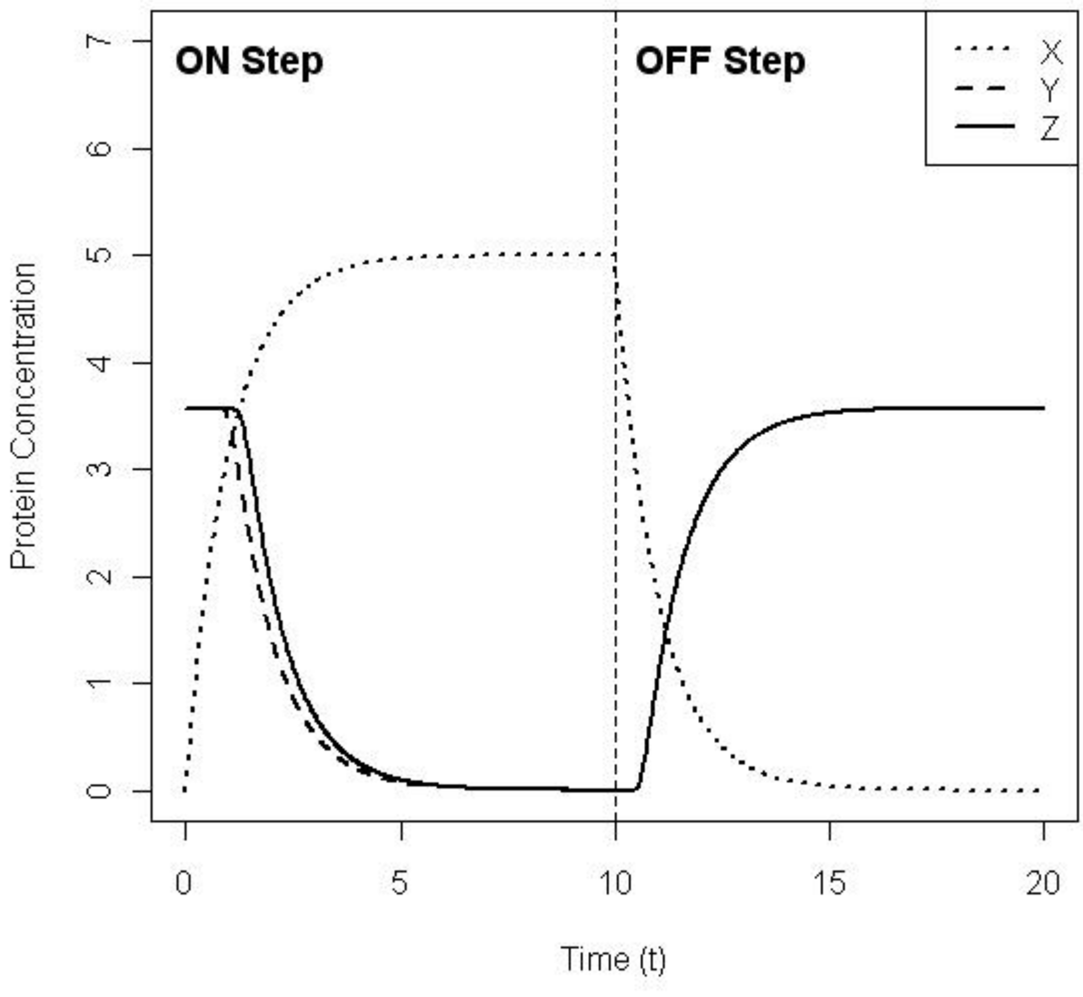

Figure 3.2: Dynamics of C2-FFL with OR gate at ON and OFF step.

3.2 Dynamics of C3-FFL with AND/OR gate

In C3-FFL with AND gate, the production of protein *Z* is activated in the absence of both X^* and Y^* (Figure 3.3). At the ON step, when X^* exceeds K_{xz}, it results in immediate stoppage of the production of protein *Z*. Thus, the concentration of protein *Z* decreases with its degradation/dilution rate and there is no delay of repression. Simultaneously, the production of protein *Y* is activated by X^* when X^* exceeds K_{xy}.

At the OFF step of C3-FFL with AND gate, the activation signal for X^* is switched off and X^* decreases. When X^* falls below K_{xz} and K_{xy}, the production of protein *Y* is inactivated and decreases while production of protein *Z* is not resumed due to the presence of its repressor. Lately, when Y^* falls below K_{yz}, protein *Z* begins to be produced; production is delayed until both X^* and Y^* fall below K_{xz} and K_{yz}, respectively.

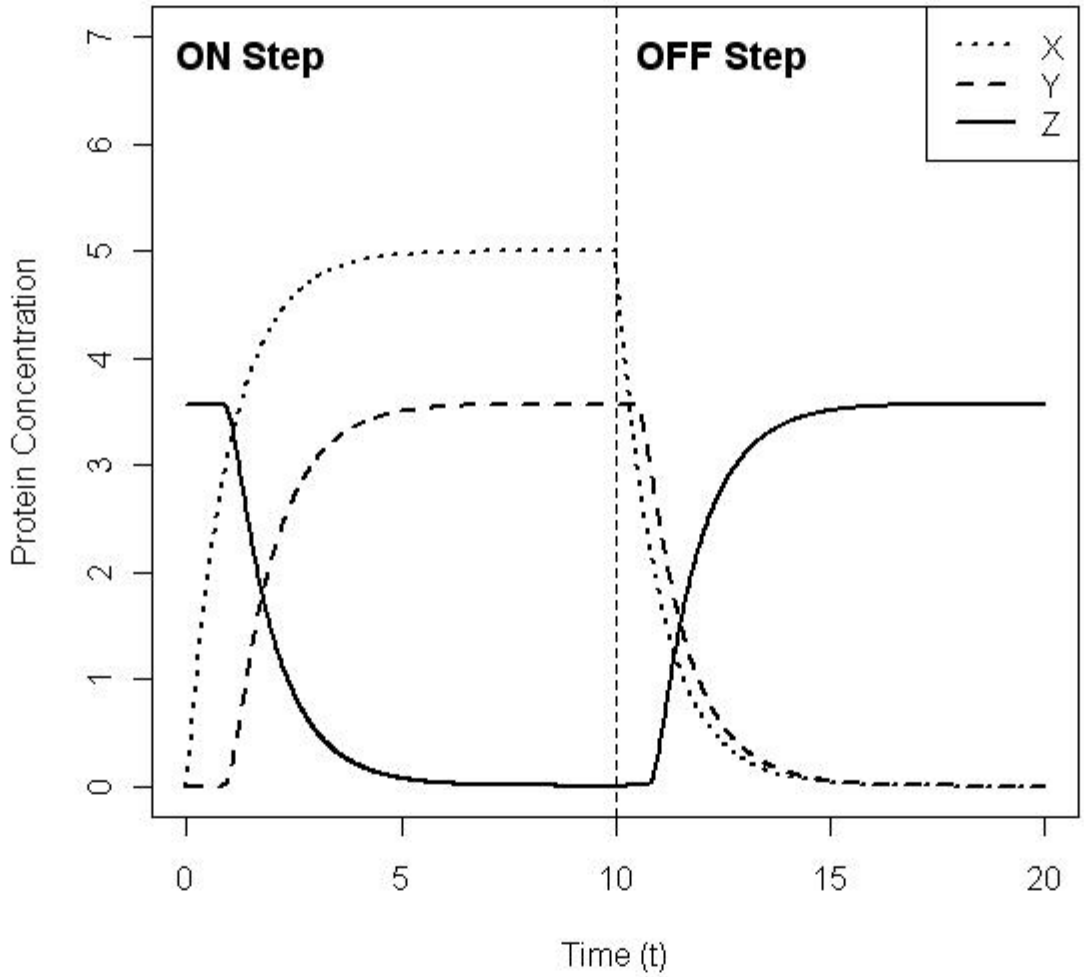

Figure 3.3: Dynamics of C3-FFL with AND gate at ON and OFF step.

The dynamics of C3-FFL with OR gate is similar to the dynamics of C3-FFL with AND gate (Figure 3.4). At the ON step, the concentration of X^* is sufficient enough to repress the production of protein Z regardless of the absence of Y^*. Thus, the production of protein Z is switched off without delay. At the OFF step, either X^* or Y^* falls below the repression threshold when the production of protein Z resumes. Thus, there is delay in resuming protein Z production.

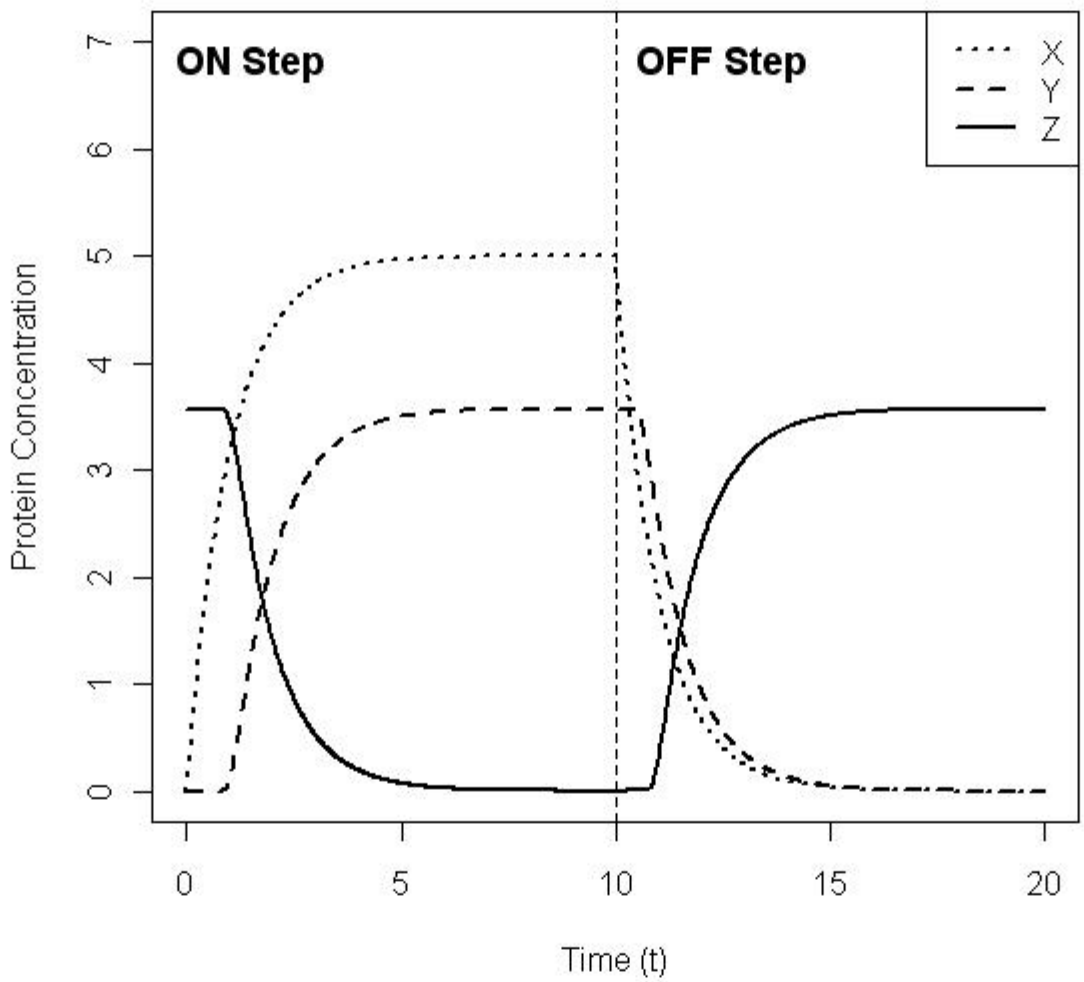

Figure 3.4: Dynamics of C3-FFL with OR gate at ON and OFF step.

3.3 Dynamics of C4-FFL with AND/OR gate

In C4-FFL with AND gate, the production of protein *Z* is activated in the presence of X^* and absence of Y^* (Figure 3.5). At the ON step, the concentration of X^* exceeds K_{xy} and results in cease of production of protein *Y*. To show the effect of repression of protein *Z* production by Y^*, protein *Z* was given a starting concentration of 3.5. When the concentration of Y^* has not reduced below K_{yz}, the concentration of protein Z decreases until concentration of Y^* falls below the K_{yz}. In the absence of Y^*, the concentration of X^*, now above K_{xz}, is able to resume the production of protein Z before it decreases to zero. Thus, there is delay in the production of protein Z.

At the OFF step of C4-FFL with AND gate, the activation signal for X^* is switched off and X^* decreases. When X^* falls below K_{xz} and K_{xy}, the production of protein Y is resumed while production of protein Z decreases. Lately, when Y^* falls below K_{yz}, the production of protein Z is delayed when both X^* and Y^* are absent. Thus, there is no delay of repression of protein Z production.

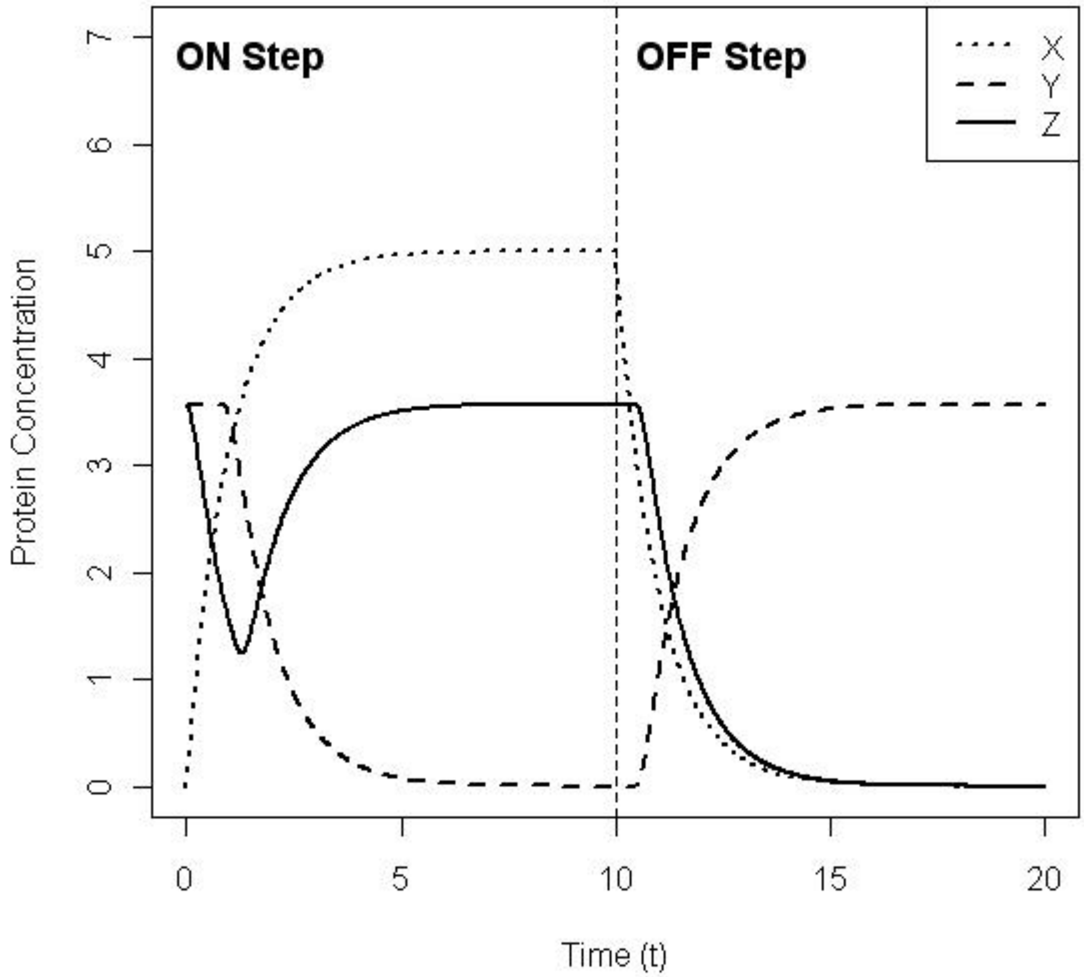

Figure 3.5: Dynamics of C4-FFL with AND gate at ON and OFF step.

Figure 3.6 shows that the dynamics of C4-FFL with OR gate is similar to the dynamics of C4-FFL with AND gate. Similar to the case of the dynamics of C3-FFL with AND and OR gate, the Boolean input gate has no effect on the response time pattern in C4-FFL with the presence and absence of activation signal for protein *X*. Thus, there is delay of activation of protein *Z* production at the ON step and no delay of repression of protein *Z* production at the OFF step in C4-FFL.

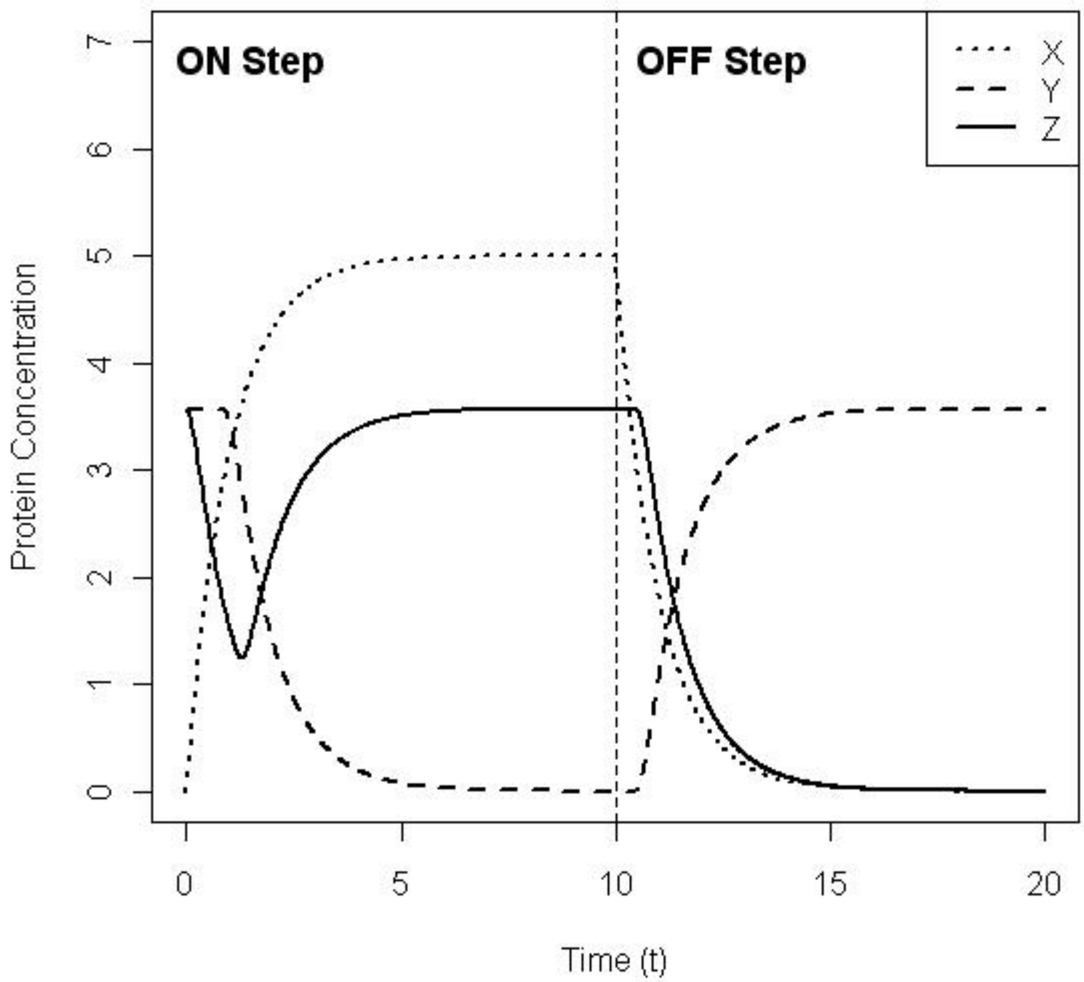

Figure 3.6: Dynamics of C4-FFL with OR gate at ON and OFF step.

3.4 Dynamics of I2-FFL with AND gate

In I2-FFL with AND gate, the production of protein Z is activated when both X^* and Y^* are absent (Figure 3.7). At the ON step, X^* exceeds K_{xy} and K_{xz}, and results in the cease of the production of protein Y and Z. Thus, there is no delay of repression of protein Z production.

At the OFF step of I2-FFL with AND gate, the activation signal for X^* is switched off and X^* decreases. It shows interesting dynamics pattern which is not found in all coherent FFL, the acceleration in production of protein Z at certain time point. When X^* falls below the K_{xz} and both X^* and Y^* are absent, the activation of protein Z production is enhanced and accelerated to the concentration higher than its steady-state. When the production of protein Y is resumed and exceeds K_{yz}, the production of protein Z is repressed and decreases to its steady-state. Thus, there is acceleration in protein Z production and its concentration remains at steady-state when it is repressed by Y^*.

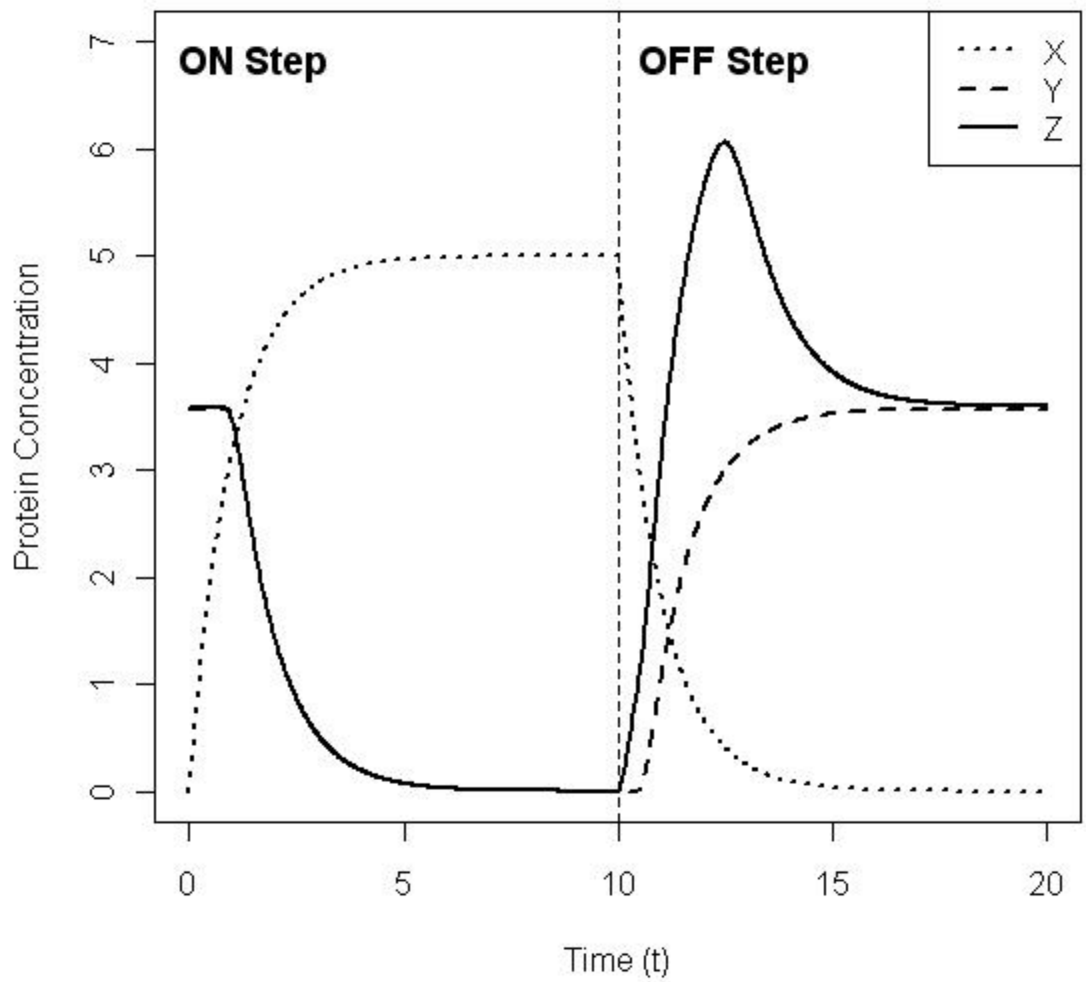

Figure 3.7: Dynamics of I2-FFL with AND gate at ON and OFF step.

3.5 Dynamics of I3-FFL with AND gate

In I3-FFL with AND gate, the production of protein *Z* is activated in the absence of X^* and in the presence of Y^* (Figure 3.8). At the ON step, X^* exceeds K_{xy} and K_{xz}, and results in the cease of the production of protein *Z* without depending on the concentration of Y^*. Thus, there is no delay of repression of protein *Z* production.

At the OFF step of I3-FFL with AND gate, the activation signal for X^* is switched off and X^* decreases. Despite the fact that there is reduction of response time in production of protein *Z*, its concentration during production time does not accelerate. Protein *Y* production is repressed when X^* falls below K_{xy}.

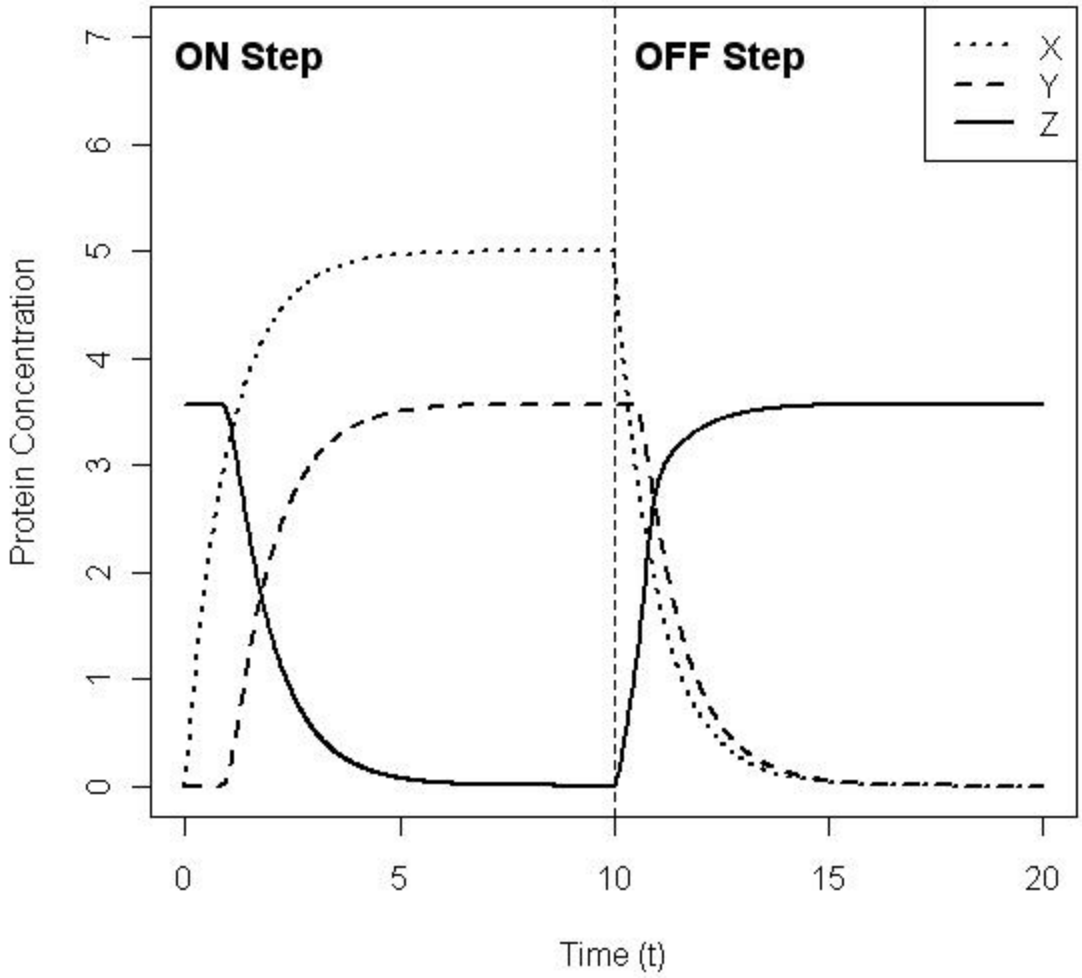

Figure 3.8: Dynamics of I3-FFL with AND gate at ON and OFF step.

To investigate the dependency of gene *Y* promoter activity on accelerating pattern of protein Z production in I3-FFL with AND gate at the OFF step, the promoter activity of gene *Y* was given values of 5, 7.5 and 10 and their dynamics were simulated to obtain the production of protein *Z* at the OFF step with different gene *Y* promoter activity (Figure 3.9). The simulation result shows that there is acceleration in protein *Z* production when the gene

Y promoter activity is the strongest at 10. Thus, it indicates that the strength of gene *Y* promoter contributes to the accelerating pattern in protein *Z* production at the OFF step.

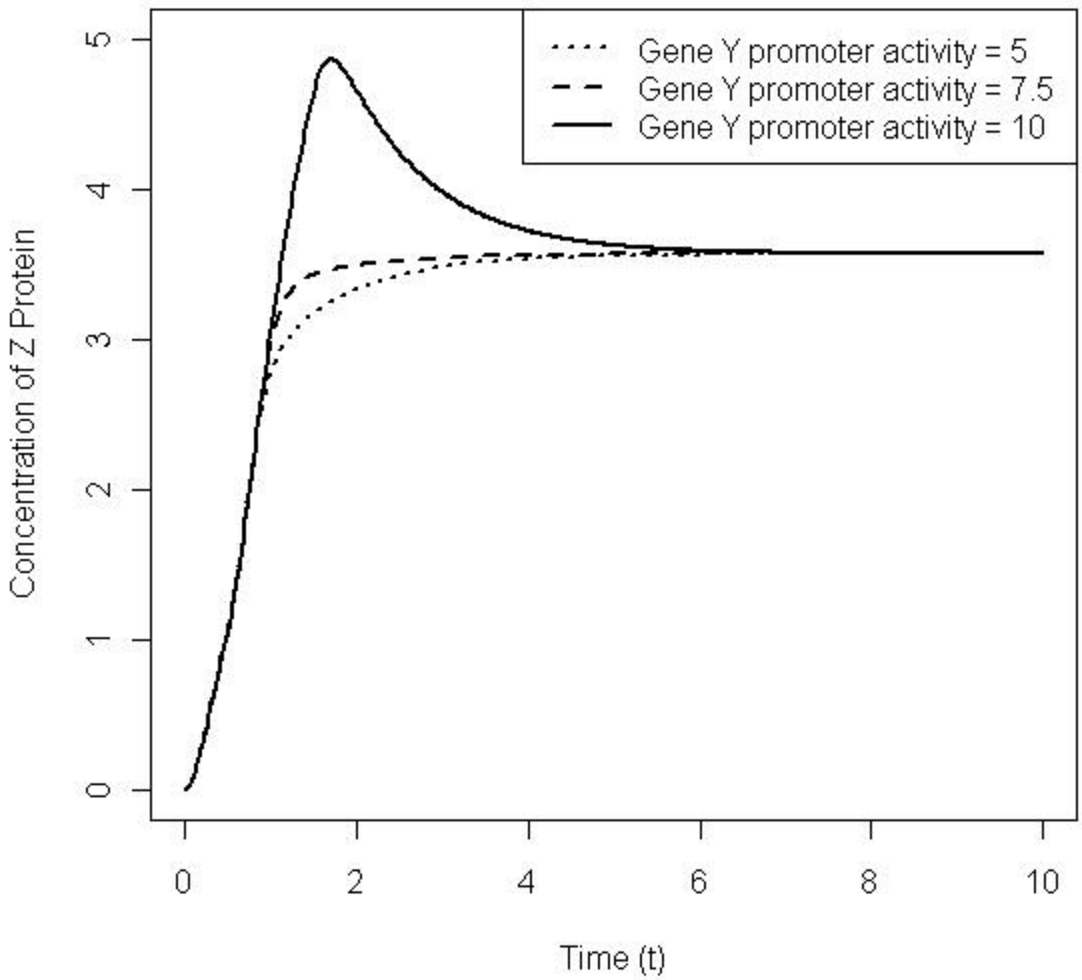

Figure 3.9: The Effect of gene *Y* promoter activity on acceleration of protein *Z* production in I3-FFL with AND gate at OFF step.

3.6 Dynamics of I4-FFL with AND gate

The dynamics of I4-FFL with AND gate is opposite to the dynamics of I3-FFL with AND gate in term of the ON or OFF step. In I4-FFL with AND gate, the production of protein *Z* is activated in the presence of X^* and Y^* (Figure 3.10). The production of protein *Y* is repressed by X^*. At the ON step, X^* exceeds K_{xz} and results in inactivation of protein *Z* production. When Y^* does not fall below the K_{yz}, the production of protein *Z* is enhanced and the response time is shortened. Nevertheless, there is no acceleration observed in protein *Z* production.

At the OFF step of I4-FFL with AND gate, the activation signal for X^* is switched off and X^* decreases. At the ON step, X^* falls below K_{xy} and K_{xz}, and results in the resume of protein *Y* production and the cease of the production of protein *Z* without depending on the concentration of Y^*. Thus, there is no delay of repression of protein *Z* production.

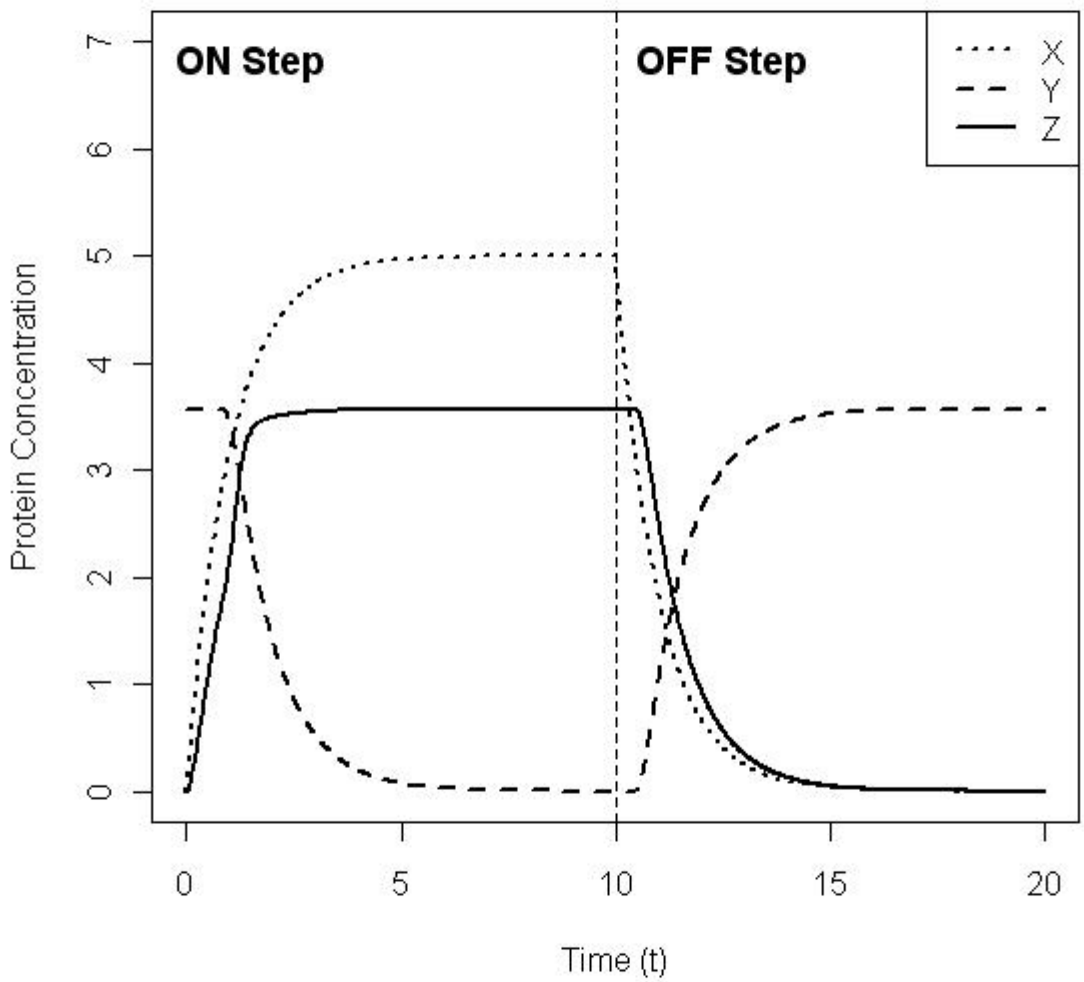

Figure 3.10: Dynamics of I4-FFL with AND gate at ON and OFF step.

To investigate the dependency of gene *Y* promoter activity on accelerating pattern of protein *Z* production in I4-FFL with AND gate at the ON step, the promoter activity of gene *Y* was given values of 5, 7.5 and 10 and their dynamics were simulated to obtain the production of protein *Z* at the OFF step with different gene *Y* promoter activity (Figure 3.11). The simulation result shows that there is an acceleration of protein *Z* production when the gene *Y* promoter activity is at 7.5 or 10. Thus, similar to I3-FFL with AND gate at the OFF step, it indicates that the strength of gene *Y* promoter contributes to the accelerating pattern in protein *Z* production at the ON step.

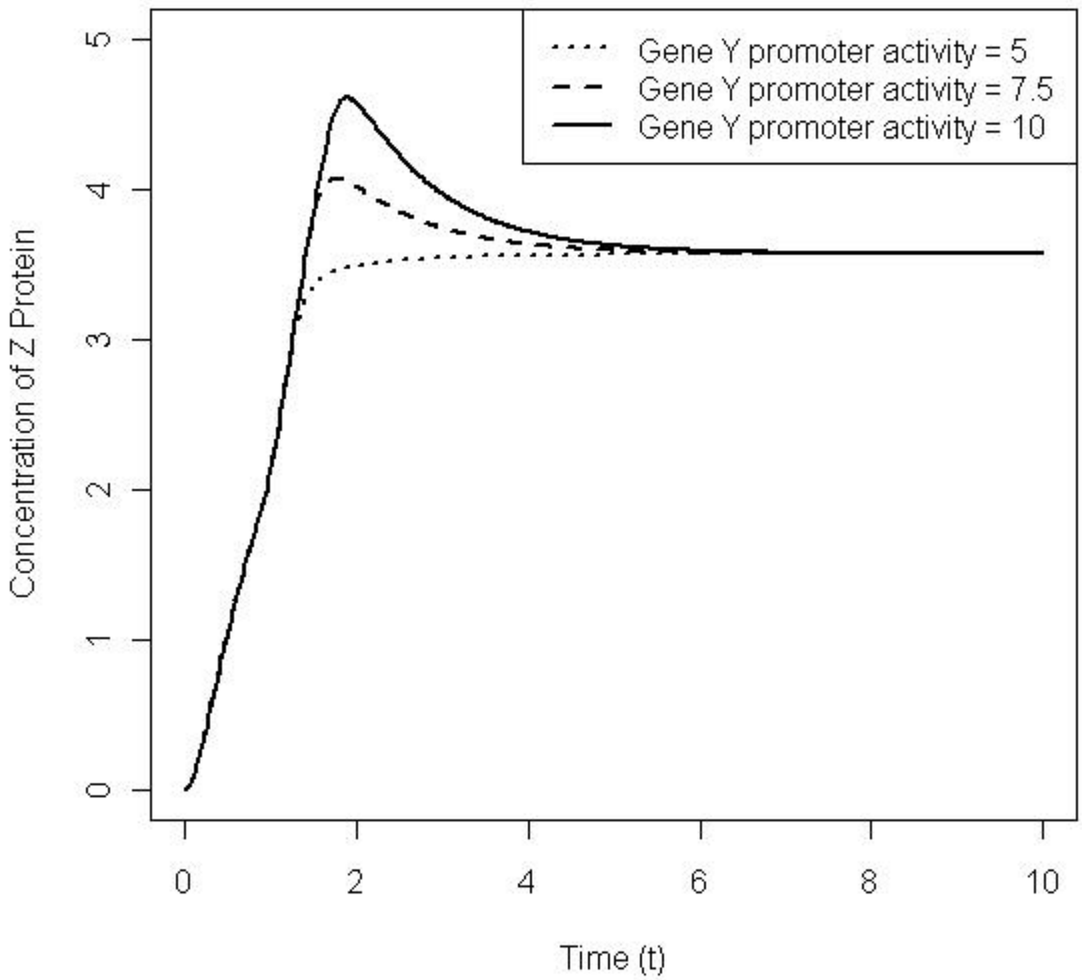

Figure 3.11: The Effect of gene *Y* promoter activity on acceleration of protein *Z* production in I4-FFL with AND gate at ON step.

3.7 Peculiar FFL transcription networks in *E. coli*

The result of mining the RegulonDB showed that 84 of 1702 TF-operon interactions were identified as being involved. In total, 28 FFL were identified in transcription networks of *E. coli*; nine of them were identified as belonging to peculiar FFL. No I2-FFL and I4-FFL were identified from the datasets of TF-operon interactions.

Table 3.1: List of peculiar FFL associated with various biological functions extracted from TF-operon interactions, RegulonDB.

X	Y	Z	FFL Type	Biological functions
crp	*malI*	*malXY*	C4-FFL	Maltose utilisation
fnr	*arcA*	*cydAB*	I3-FFL	Anaerobic respiration
fnr	*arcA*	*cyoABCDE*	C3-FFL	Anaerobic respiration
fnr	*arcA*	*icdA*	C3-FFL	Anaerobic respiration
fnr	*arcA*	*ndh*	I3-FFL	Anaerobic respiration
fnr	*arcA*	*nuoABCEFGHIJKLMN*	C3-FFL	Anaerobic respiration
fnr	*arcA*	*sdhCDAB_b0725_sucABCD*	C3-FFL	Anaerobic respiration
ihf	*ompR_envZ* (*ompB*)	*ompC*	C2-FFL	Osmoregulatory response
ihf	*ompR_envZ* (*ompB*)	*ompF*	C2-FFL	Osmoregulatory response