Synthesis of Clock Signal from Genetic Oscillator

Chia-Hua Chuang and Chun-Liang Lin

Department of Electrical Engineering, National Chung Hsing University,
Taichung 402, Taiwan
`chunlin@dragon.nchu.edu.tw`

Abstract. This paper attempts to design a genetic frequency synthesizer circuit with counter to synthesize a clock signal whose frequency is a multiple of that of an existing synthetic genetic oscillator. A genetic waveform-shaping circuit constructed by Buffers in series is used to reshape a genetic oscillation signal into a pulse-width-modulated (PWM) signal with different duty cycle. Design of the Buffers and accompanied genetic logic gates is based on the use of the real genetic structural genetic algorithm. By assembling different PWM signals, a series of clock pulses is synthesized as the rising and falling edges of the desired clock signals triggering the counter and the clock signal with the integer multiple of base frequency is generated from the same oscillation signal. Simulation results show that the proposed genetic frequency synthesizer circuit is effective to realize a variety of genetic clocks.

Keywords: circuit synthesis, biology, repressilator, logic gate, synthesizer.

1 Introduction

Synthetic biology is developed to construct an artificial genetic circuit using the approaches of mathematics and engineering [1-3]. Several synthetic genetic circuits have successfully been built to achieve the basic functions, e.g. genetic oscillator generates a periodic oscillation signal and genetic logic circuit performs biological logical computation which is an important device for constructing the more complicated bio-computers [4-7]. Based on a bottom-up approach, more complicated bio-computing processes can be expected to perform specific functions, like very-large-scale integration (VLSI) circuits in electronic systems.

There are many engineering methods proposed to convert a synthetic design problem into a tracking optimization problem. Based on those methods, a class of synthetic genetic circuits is implemented for new tasks. In [8], a robust design approach based on H_∞ optimization theory is proposed to construct a robust synthetic genetic oscillator with the desired sustained periodic oscillation behavior under the stochastic perturbational environment. For embedding the synthetic genetic circuit into the host cell, a combined parameter and structure optimization formulation has been attempted. A real structural genetic algorithm (RSGA) has been applied to synthesize a class of genetic logic circuits with the minimal number of genes while ensuring acceptable performance [9, 10]. A variety of clock signals is synthesized

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from the base-frequency oscillation signal using genetic frequency synthesizer circuits [11, 12]. One may refer, for example, to [13] for other applications oriented from the RSGA. In which, comparison of a class of GA-based algorithms can also be found.

This paper presents a genetic frequency synthesizer circuit with counter to generate a genetic clock signal based on the existing synthetic genetic oscillator. Frequency of the clock signal is multiple to that of the genetic oscillator. A genetic waveform-shaping circuit is firstly constructed by Buffers in series to reshape genetic oscillation into an approximate clock signal with explicit discrimination between low and high logic levels. To regulate the different threshold levels of a "Buffer", a pulse-width-modulated (PWM) signal with different duty cycles in a sinusoidal cycle can be generated on the same oscillation signal. Based on the specific feature, the rising and falling edges of an ideal clock signal can be determined and a series of clock pulse signals is obtainable by assembling the different PWM signals. Appling the generated clock pulse signal to trigger a 1-bit genetic counter constructed by JK flip-flop, the desired genetic clock with the integer number of frequency of genetic oscillator can be realized.

Different from [11], an edge-triggered genetic frequency synthesizer circuit is designed to construct the clock signal with a multiple of frequency of the genetic oscillator. Simulation results *in silico* show performance of the synthetic genetic clock with base frequencies while operating at a genetic oscillator.

2 Model Description

In biological systems, the synthetic genetic network with L genes is described by the following nonlinear Hill differential equation [6, 11, 12]

$$\dot{p}_i = \alpha_i f_i(u) - \beta_i p_i + \alpha_{0,i},\ i = 1,\ldots,L \tag{1}$$

where p_i is the concentration of protein for gene i, α_i, β_i and $\alpha_{0,i}$ are, respectively, the synthesis, degradation and basal rates, $f_i(\cdot)$ denotes the promoter activity function used to describe the nonlinear transcriptional logic reactions, and u is the concentration of transcription factor (TF) from other gene's produces or inducers to control the gene expression.

For a gene with an operator site, the promoter activity functions for the genetic logic NOT and the Buffer are described, respectively, as

$$f_{\mathrm{NOT}}(u) = \frac{1}{1+\left(\dfrac{u}{K}\right)^n} \tag{2}$$

and

$$f_{\text{Buffer}}(u) = \frac{\left(\frac{u}{K}\right)^n}{1+\left(\frac{u}{K}\right)^n} \tag{3}$$

where f_{NOT} and f_{Buffer} are the promoter activity functions for logic NOT and Buffer, respectively, u is the concentration of a repressor or activator TF, n is the Hill coefficient, and K is the Hill constant.

For a gene with two operator sites, the promoter activity functions for the genetic logic AND, OR, NAND, NOR and XOR gates are described as

$$f_{\text{AND}}(u_1,u_2) = \frac{\left(\frac{u_1}{K_1}\right)^{n_1}\left(\frac{u_2}{K_2}\right)^{n_2}}{1+\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}+\left(\frac{u_1}{K_1}\right)^{n_1}\left(\frac{u_2}{K_2}\right)^{n_2}} \tag{4}$$

$$f_{\text{OR}}(u_1,u_2) = \frac{\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}+\left(\frac{u_1}{K_1}\right)^{n_1}\left(\frac{u_2}{K_2}\right)^{n_2}}{1+\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}+\left(\frac{u_1}{K_1}\right)^{n_1}\left(\frac{u_2}{K_2}\right)^{n_2}} \tag{5}$$

$$f_{\text{NAND}}(u_1,u_2) = \frac{1+\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}}{1+\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}+\left(\frac{u_1}{K_1}\right)^{n_1}\left(\frac{u_2}{K_2}\right)^{n_2}} \tag{6}$$

$$f_{\text{NOR}}(u_1,u_2) = \frac{1}{1+\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}+\left(\frac{u_1}{K_1}\right)^{n_1}\left(\frac{u_2}{K_2}\right)^{n_2}} \tag{7}$$

and

$$f_{\text{XOR}}(u_1,u_2) = \frac{\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}}{1+\left(\frac{u_1}{K_1}\right)^{n_1}+\left(\frac{u_2}{K_2}\right)^{n_2}+\left(\frac{u_1}{K_1}\right)^{n_1}\left(\frac{u_2}{K_2}\right)^{n_2}} \tag{8}$$

where f_{AND}, f_{OR}, f_{NAND}, f_{NOR} and f_{XOR} are, respectively, the promoter activity functions for logic AND, OR, NAND, NOR and XOR reactions, u_1 and u_2 are the concentrations of two repressor or activator TFs, K_1 and K_2 are Hill constants for

u_1 and u_2, respectively, and n_1 and n_2 are the corresponding Hill coefficients. Determination of the key parameters in (4) to (8) was obtained using the RSGA [10].

3 Synthesis of Clock Signal

3.1 Realization of Genetic Waveform-Shaping Circuit

A waveform-shaping circuit in electronics is constructed to convert the oscillation signal to the clock signal with the explicit logic edge. As the red line shown in Fig. 1, the input signal is mapped to the low level when it is less than a threshold level y_T while it is mapped to the high level when it exceeds the threshold level. In reality, this curve in biological systems doesn't exist. A more reasonable input and output (I/O) characteristic curve of a sigmoid function (blue line in Fig. 1) is thus considered. There are two operational regions: saturation region and transition region. The input signal in the saturation region is mapped to the high level or the low level. In the transition region, the gain in the operation point y_T must be larger than (normalized) 1 to ensure that the input will be amplified when it is larger than the threshold level and decayed when it is less than the threshold level. By connecting a series of sigmoid functions, the input signal will gradually reach the saturation region and stay at the high or low level.

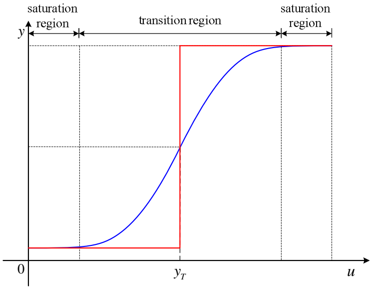

Fig. 1. I/O characteristic curve

According to this idea, we use several cascaded Buffers to realize the genetic waveform-shaping circuit described by [11, 12]

$$\dot{p}_{B_k} = \alpha_{B_k} f_{\text{Buffer},k}\left(u_k, K_k, n_k\right) - \beta_{B_k} p_{B_k} + \alpha_{B_{0,k}},\ k = 1,\dots,M \tag{9}$$

and the steady-state solution is easily obtained as

$$p_{B_{k,ss}} = \frac{\alpha_{B_k}}{\beta_{B_k}} f_{\text{Buffer},k}\left(u_k, K_k, n_k\right) + \frac{\alpha_{B_{0,k}}}{\beta_{B_k}} \tag{10}$$

where p_{B_k} is the output concentration of the *k*th Buffer, $p_{B_{k,ss}}$ denotes its steady-state concentration, u_k, K_k and n_k are respectively the input concentration, Hill constant, and Hill coefficient of the *k*th Buffer, and α_{B_k}, β_{B_k} and $\alpha_{B_{0,k}}$ are, respectively, synthesis, decay and basal rates. The second term in the right side of (10) is minimal level and α_{B_k}/β_{B_k} is the difference between the minimal level and the maximal level. The output concentration of the Buffer is the half maximal output concentration when the input concentration equals K_k and K_k refers to the threshold level y_T.

The gain in the operation point K_k is obtained by

$$A_{B_k} = \left.\frac{\partial p_{B_{k,ss}}}{\partial u_k}\right|_{u_k=K_k} = \frac{\alpha_{B_k} n_k}{4\beta_{B_k} K_k} \tag{11}$$

where A_{B_k} is the gain of the *k*th Buffer. It is observed that the gain is proportional to the Hill coefficient n_k and is inversely proportional to the Hill constant K_k at the operation point $u_k = K_k$. To achieve the genetic waveform-shaping circuit design, the necessary condition of the gain at the operation point K_k should be more than 1.

In each stage, the corresponding input signals and threshold levels are given by

$$u_k = \begin{cases} A\sin\left(\omega_0 t + \varphi\right) + y_{d,0}, & k = 1 \\ p_{B_{k-1}}, & k > 1 \end{cases} \tag{12}$$

and

$$K_k = \begin{cases} y_T, & k = 1 \\ \dfrac{\alpha_{B_{k-1}} + \alpha_{B_{0,k-1}}}{2\beta_{B_{k-1}}}, & k > 1 \end{cases} \tag{13}$$

where A, ω_0, φ and $y_{d,0}$ are, respectively, amplitude, basal frequency, phase, and base level of the desired oscillation signal. In the first stage, the input signal is oscillation signal produced by the genetic oscillator and described by a sinusoidal function. In the next stage, the input signal and the threshold level are respectively the output and the half maximal output level in the previous stage. Figure 2 shows topology of the designed genetic waveform-shaping circuit. The produced protein of the first gene activates the transcription of the second gene whose production activates the next gene.

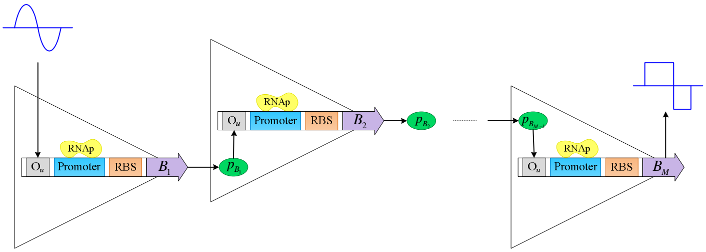

Fig. 2. Topology of the genetic waveform-shaping circuit

3.2 Regulation of Threshold Level

To regulate different threshold levels in (13), one can generate the PWM signal with different duty cycle defined by

$$D = \frac{T_{\text{on}}}{T_0} \times 100\% \tag{14}$$

where D is the duty cycle, T_0 is the basal period of oscillation signal with $2\pi/\omega_0$ and T_{on} is the period of logic high in a basal period. For the different duty cycle, the corresponding threshold value is selected by

$$y_T = A\sin\left(\omega_0 t + \varphi\right) + y_{d,0},\ t = t_h \pm \frac{T_{\text{on}}}{2} \tag{15}$$

and $t_h = \dfrac{1}{\omega_0}\sin^{-1}(1) - \dfrac{\varphi}{\omega_0}$, $t_h \in [0 \quad T_0]$. Clearly, the PWM signal has 50% duty cycle when $y_T = y_{d,0}$.

3.3 Design of Genetic Frequency Synthesizer Circuit

Frequency synthesizer is designed to generate an output signal whose frequency is a multiple of that of input signal in electronics. Based on the feature, one can generate the clock pulses with frequency multiple to that of the genetic oscillator by using the genetic waveform-shaping circuit mentioned above. To construct a clock signal with *N*-fold frequency of genetic oscillator, a series of clock pulses should be stimulated by the threshold levels:

$$y_{T_\varepsilon} = A\sin\left(\omega_0 t_\varepsilon + \varphi\right) + y_{d,0},\ \varepsilon = 1,\ldots,N+1 \tag{16}$$

with $t_\varepsilon = t_h + \dfrac{T_0}{2N}(\varepsilon - 1)$, $T_0 = \dfrac{2\pi}{\omega_0}$, where y_{T_ε} is the value of threshold level for the synthesis of frequency synthesizer and t_h in (15). For example, the clock pulses become activated in the threshold levels $y_{d,0}+A$ and $y_{d,0}-A$ for the clock signal with 50% duty cycle shown in Figs. 3(a) and 3(b). For the clock signal with double basal frequency, the clock pulses become simulated in the threshold levels $y_{d,0}+A$, $y_{d,0}-A$, and $y_{d,0}$. To generate the clock pulse in the threshold level $y_T = y_{d,0}$, a genetic logic XOR gate is used to combine two PWM signals with threshold levels $y_{d,0}+\Delta y_T$ and $y_{d,0}-\Delta y_T$ where Δy_T is a small variation.

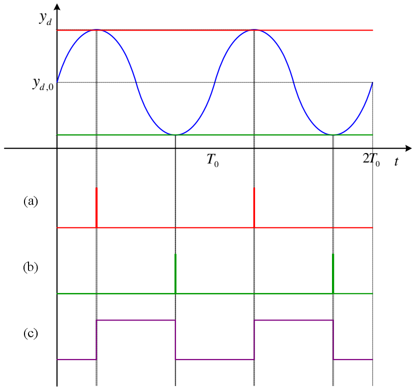

Fig. 3. Ideal signals for the design of clock signal with 50% duty cycle. (a) clock pulse in $y_T = y_{d,0}+A$; (b) clock pulse in $y_T = y_{d,0}-A$; and (c) clock signal.

After generating the clock pulses via the proposed genetic waveform-shaping circuit, the 1-bit genetic counter shown in Fig. 4 is triggered by the clock pulse to synthesize the genetic clock with the multiple frequencies to the genetic oscillator. Figure 5 displays the topology of 1-bit genetic counter with the model constructed as

$$\begin{aligned}
\dot{p}_W &= \alpha_W f_{\mathrm{AND}}\left(p_K, p_{CLK}\right) - \beta_W p_W, \\
\dot{p}_V &= \alpha_V f_{\mathrm{AND}}\left(p_J, p_{CLK}\right) - \beta_V p_V, \\
\dot{p}_R &= \alpha_R f_{\mathrm{AND}}\left(p_W, p_Q\right) - \beta_R p_R, \\
\dot{p}_S &= \alpha_S f_{\mathrm{AND}}\left(p_V, p_{\bar{Q}}\right) - \beta_S p_S, \\
\dot{p}_Q &= \alpha_Q f_{\mathrm{NOR}}\left(p_R, p_{\bar{Q}}\right) - \beta_Q p_Q, \\
\dot{p}_{\bar{Q}} &= \alpha_{\bar{Q}} f_{\mathrm{NOR}}\left(p_S, p_Q\right) - \beta_{\bar{Q}} p_{\bar{Q}}
\end{aligned} \tag{17}$$

where p_{CLK} is the clock pulse signal, $p_K = p_J = 1$, p_W, p_V, p_R, p_S, p_Q and $p_{\bar{Q}}$ are respectively the concentrations of productions of the genes, α_W, α_V, α_R, α_S, α_Q and $\alpha_{\bar{Q}}$ are synthesis rates, and β_W, β_V, β_R, β_S, β_Q and $\beta_{\bar{Q}}$ are decay rates.

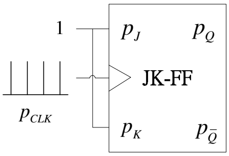

Fig. 4. A 1-bit genetic counter circuit with the rising edge-triggered JK flip-flop

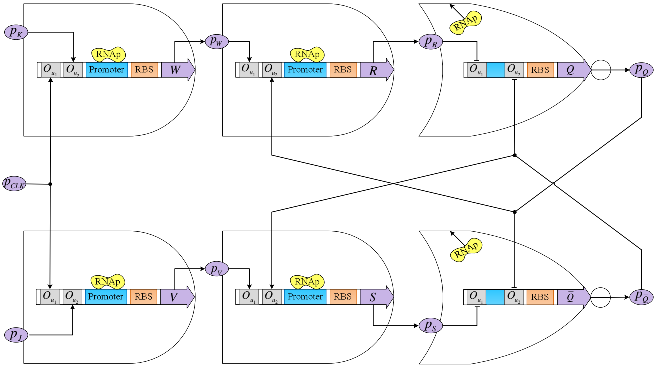

Fig. 5. Topology of the rising edge-triggered JK flip-flop

4 Simulation Results

An example of the existing synthetic genetic oscillator, known as a repressilator, is illustrated to confirm performance of the designed genetic logic circuit. The dynamic model of the repressilator [4] is given by

$$\begin{aligned} \dot{p}_{lacI} &= 0.2877\frac{1}{1+p_{cI}^4} - 0.0974\,p_{lacI}, \quad p_{lacI}(0) = 0.7, \\ \dot{p}_{tetR} &= 0.2877\frac{1}{1+p_{lacI}^4} - 0.0974\,p_{tetR}, \; p_{tetR}(0) = 1.2, \\ \dot{p}_{cI} &= 0.2877\frac{1}{1+p_{tetR}^4} - 0.0974\,p_{cI}, \quad p_{cI}(0) = 1.7 \end{aligned} \tag{18}$$

where p_{lacI}, p_{tetR} and p_{cI} are, respectively, the produced proteins of the repressor genes $lacI$, $tetR$ and cI. This oscillation signal has the basal period $T_0 = 39$ sec, the amplitude $A = 0.63$, and the base level $y_{d,0} = 1.1731$. Assume that p_{cI} is the oscillation input with concentration response shown in Fig. 6.

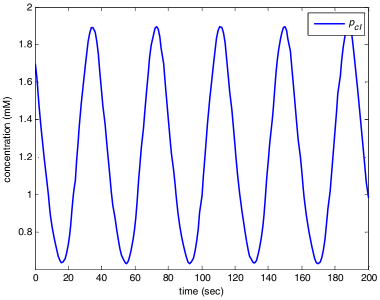

Fig. 6. Response of concentration of p_{cI}

To synthesize the clock pulse in the threshold level $y_{d,0}+A$, the designed genetic waveform-shaping circuit is obtained as

$$\begin{aligned}
&\dot{p}_{B_1}=f_{\text{Buffer}}\left(p_{cI},1.7462,4\right)-p_{B_1},\ \dot{p}_{B_2}=f_{\text{Buffer}}\left(p_{B_1},0.5,4\right)-p_{B_2},\\
&\dot{p}_{B_3}=f_{\text{Buffer}}\left(p_{B_2},0.5,4\right)-p_{B_3},\ \dot{p}_{B_4}=f_{\text{Buffer}}\left(p_{B_3},0.5,4\right)-p_{B_4},\\
&\dot{p}_{B_5}=f_{\text{Buffer}}\left(p_{B_4},0.5,4\right)-p_{B_5},\ \dot{p}_{B_6}=1.1073f_{\text{Buffer}}\left(p_{B_5},0.5,4\right)-p_{B_6}
\end{aligned}\tag{19}$$

where p_{B_6} is the clock pulse signal. This circuit consists of six cascaded Buffers. In the first stage, the threshold level 1.7462 is designed. According to (13), the threshold level 0.5 for the second to the sixth Buffers is chosen. To compensate the output of maximal level, the appropriate rate constants for the last Buffer is selected by (10). The concentration response of the designed clock pulse signal is shown in Fig. 7.

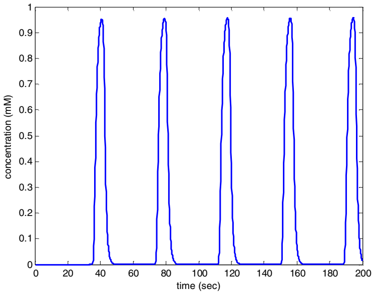

Fig. 7. Response of the designed clock pulse signal in the threshold level $y_{d,0}+A$

Similarly, the genetic waveform-shaping circuit for design of the clock pulse in the threshold level $y_{d,0}-A$ is obtained as

$$\begin{aligned}
&\dot{p}_{B_1} = f_{\text{Buffer}}\left(p_{cI}, 0.6852, 4\right) - p_{B_1},\ \dot{p}_{B_2} = f_{\text{Buffer}}\left(p_{B_1}, 0.5, 4\right) - p_{B_2}, \\
&\dot{p}_{B_3} = f_{\text{Buffer}}\left(p_{B_2}, 0.5, 4\right) - p_{B_3},\ \dot{p}_{B_4} = f_{\text{Buffer}}\left(p_{B_3}, 0.5, 4\right) - p_{B_4}, \\
&\dot{p}_{B_5} = f_{\text{Buffer}}\left(p_{B_4}, 0.5, 4\right) - p_{B_5},\ \dot{p}_{B_6} = 1.0867 f_{\text{Buffer}}\left(p_{B_5}, 0.5, 4\right) - p_{B_6}, \\
&\dot{p}_{B_7} = f_{\text{NOT}}\left(p_{B_6}, 0.5, 4\right) - p_{B_7}
\end{aligned} \tag{20}$$

where p_{B_7} is the desired clock pulse signal. This circuit is constructed by six cascaded Buffers and a NOT gate. In the first stage, the threshold level 0.6852 is designed. According to (13), the threshold level 0.5 for the second to the sixth Buffers is chosen. Figure 8 shows the concentration response of the designed clock pulse.

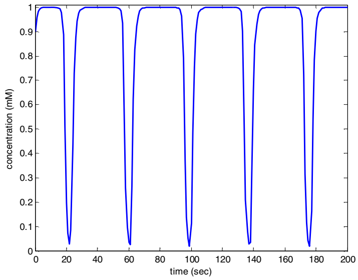

Fig. 8. Response of the designed clock pulse signal in the threshold level $y_{d,0} - A$

Using the logic AND gate in (4) with $\alpha = \beta = 1$, $n_1 = n_2 = 4$, and $K_1 = K_2 = 0.5$ to integrate the designed clock pulses in (19) and (20), the clock pulse with double base-frequency of the genetic oscillator in (18) is synthesized. By the clock signal to trigger the counter circuit in (17) whose all rate constants are 1, all Hill constants are 0.5, and all Hill coefficients are 4, the genetic clock with base frequency is synthesized and its concentration response is shown in Fig. 9.

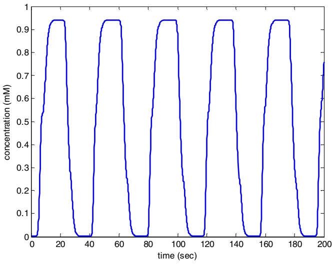

Fig. 9. Response of the designed genetic clock with base frequency

5 Conclusion

A synthetic genetic frequency synthesizer circuit with counter has been proposed to synthesize the genetic clock with the multiple to frequency of the genetic oscillator. Through applying a waveform-shaping technique, genetic oscillation is shaped to becoming an ideal logic signal. By regulating the different threshold levels, the PWM signals with different duty cycles are obtained. A genetic counter is triggered by the clock pulse generated by the proposed genetic waveform-shaping circuit to synthesize the desired genetic clock, whose frequency is a multiple to that of the genetic oscillator, is realized.

Experimental realization of the proposed network is a potential issue worthy of further investigation. In addition, assembling the clock signal with a variety of sequential genetic logic circuits to realize artificial logic functions is recommended for future research.

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