# An Artificial Regulation System for DNA-transcription: Learning from Prokaryotic Organisms

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A small molecule was labeled as an antigen in DNA during PCR. In vitro transcription reaction using the DNA as a template was regulated by specific interaction between the antigen and its monoclonal antibody.

The regulation of gene expression is a fundamental process shared by all life forms. The ability to regulate gene activity would be a valuable tool in basic and applied biological research; and could ultimately lead to new gene therapies.[1–5] Although the common form of regulation in both prokaryotes and eukaryotes is to control initiation at the level of transcription, the regulation strategy of gene expression is different between the systems.[6] In a eukaryotic system, *trans*-acting factors must bind to *cis*-acting sites in order for RNA polymerase to initiate transcription at the promoter (positive regulation). On the other hand, in prokaryotes, a *trans*-acting repressor binds to the *cis*-acting operator to turn off transcription (negative regulation). The former is coordinated by many regulatory proteins and is more complicated and sophisticated; however, the latter has the clear advantage over the eukaryotic system of being able to swiftly and flexibly change in an environment. The lac-operon is a typical genetic system of bacterial cells; actually a series of adjacent genes and regulatory elements in one small part of the *E. coli* circular chromosome.[7,8] Transcription in this system is controlled by specific interaction between repressor proteins and a *cis*-acting site called the operator. In the present study, we constructed an artificial operator and repressor using chemically modified nucleic acid and its antibody; a system modeled on the lac-operon (Figure 1).

A DNA fragment encoding the T7 promoter and luciferase gene was amplified by PCR using pRL-null (Promega) as a template. Fluorescein was immobilized on the DNA as an antigen during the same reaction using fluorescein-12-dATP (NEN Life Science Product) and Ex Taq DNA polymerase (Takara). The primers for the fragment are: GCTCACATGGCTCGAC and TCGAAGCGGCCGCTCTAGAA. The reaction mixture contained a 1:1 ratio of dATP:fluorescein-12-dATP (0.5 mM each) and 1 mM each of dCTP, dGTP, and dTTP. For DNA amplification by PCR, the thermocycler was programmed for a "hot start" at 92 °C (2 min) followed by a step-down procedure[9] (4 cycles each) with 1 min denaturation at 92 °C, 30 s annealing at 62–50 °C (annealing temperatures decreased by 4 °C every cycle), and 30 s elongation at 72 °C. The step-down procedure was followed by 20 cycles of annealing at 46 °C. The PCR product was analyzed by agarose gel electrophoresis using 1% gel according to a standard protocol. The single 1280-bp fragment was confirmed by fluorescence detections by ethidium-staining and fluorescein immobilized on the DNA. The DNA fragment was purified by Microcon® PCR (Millipore). Fluorescein-12-dATP was incorporated into the PCR-generated DNA in a ratio of fluorescein-12-dATP:dATP = 2:3 by fluorescence analysis. As a result, nearly 380 fluorescein molecules were incorporated into the DNA strand.

Gel mobility shift assay was carried out using anti-fluorescein antibody; for investigation whether fluorescein, which was immobilized on the DNA, actually acted as an antigen under physiological conditions. 600 ng of antifluorescein-HRP conjugate NEF710 (NEN Life Science Product) was added to 80 ng of modified and unmodified-DNA in PBS(−) buffer (2.68 mM KCl, 1.47 mM $KH_2PO_4$, 8.1 mM $Na_2HPO_4$ (pH7.4), 137 mM NaCl) at 4 °C for 1 h. The solutions were analyzed by agarose gel electrophoresis using 1% gel. As shown in Figure 2a, the DNA band of the modified-DNA disappeared in the presence of the antibody (lene 5). We observed that a new band, which might be a complex consisting of fluorescein-carrying DNA and anti-fluorescein antibody, was detected at the starting well by fluorescence detection of fluorescein (Figure 2b, lane 5). On the other hand, the mobility of unmodified-DNA was not

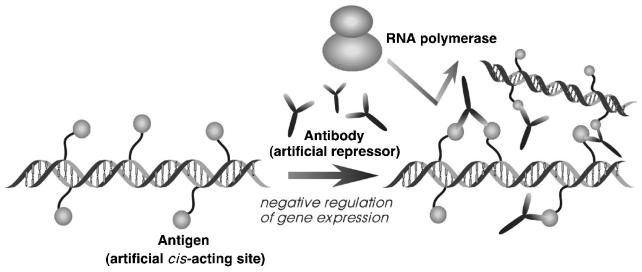


**Figure 1.** Schematic illustration of the artificial regulation system for gene expression using antigen-antibody reaction.

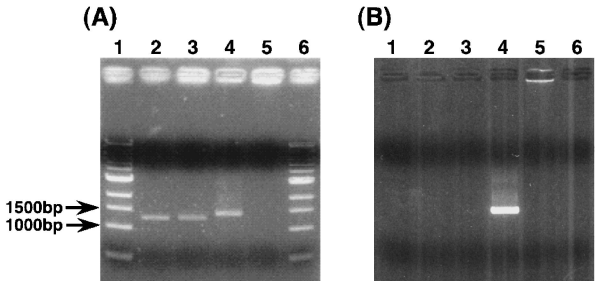


**Figure 2.** Binding of antifluorescein antibody to the antigen on the DNA. A Electrophoretic mobility band shift assay was performed using fluorescein-modified and unmodified DNA with antifluorescein-HRP conjugate. A, visualized by ethidium staining. B, fluorescence detection by fluorescein of same samples as shown in A. Lanes 1 and 6, DNA marker; lanes 2, unmodified DNA; lanes 3, unmodified DNA with antibody; lane 4, modified DNA; lane 5, modified DNA with antibody. Unmodified DNA, 80 ng; modified-DNA, 80 ng; antifluorescein-HRP conjugate, 600 ng. Samples were analysed by electrophoresis on a 1% agarose gel in TAE buffer.

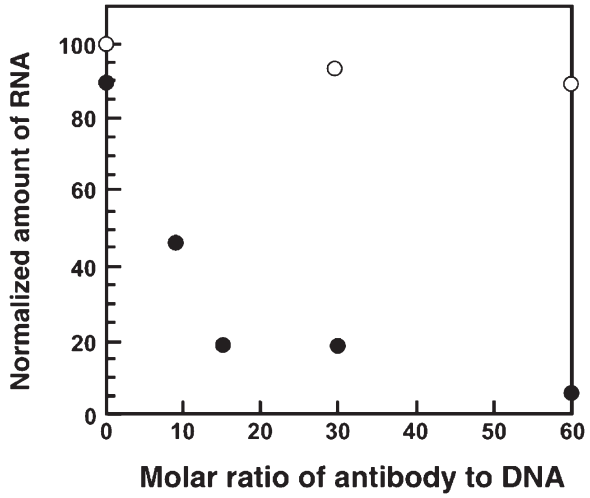


**Figure 3.** Effect of antibody on transcription reaction using antigen modified-DNA as template. ●, fluorescein-modified DNA (1.5 μg); ○, unmodified-DNA (1.2 μg). In vitro transcription reactions were carried out using RiboMAX$^{TM}$ large scale RNA production system for T7 according to standard protocol. Anti-fluorescein monoclonal antibody was used. Synthesized RNA was detected by Agilent 2100 bioanalyzer with RNA 6000 nano chip.

significantly influenced by the presence of the antibody. These results suggest that fluorescein moiety was specifically recognized by the monoclonal antibody, even if incorporated into the DNA.

To determine whether the interaction between fluorescein and its antibody on the DNA regulates transcription, the antibody was added to an in vitro transcription reaction mixture. RNA synthesis was carried out using a RiboMAX$^{TM}$ large scale RNA production system for T7 RNA polymerase (Promega). As a transcription template, the modified-(1.5 μg) and unmodified-DNA (1.2 μg) were added to the reaction mixture including rNTPs (25 mM each), enzyme mix for T7 and RNase inhibitor. The antifluorescein antibodies were added to the solutions by 9, 15, 30, and 60-fold higher amounts in mole for the template DNA, respectively. The mixtures were incubated for 2 h at 37 °C, and then template DNA was degraded by DNase (1 U) for 15 min at 37 °C. Synthesized RNA in the solution was detected by electrophoretic analysis using an Agilent 2100 bioanalyzer with a RNA 6000 nano chip. In the case of antigen-modified DNA, the amount of RNA clearly decreased with increasing dosage of antibody (Figure 3). We observed a 94% reduction in RNA production after treatment of a 60-fold higher amount of antibody. In contrast, the antibody did not affect significantly on transcription using unmodified-DNA as a template under the same conditions. Similar results were obtained from an in vitro luciferase expression experiment (data not shown). These results indicated that the antibody associated with the DNA was suppressed the transcription reaction. It is likely that the access of the RNA polymerase to the DNA is inhibited by steric hindrance of the antibody on the DNA.

In conclusion, we demonstrated a simple and effective system for regulating gene expression using the specific interaction between an antigen and its monoclonal antibody on DNA. Off course, antigen-labeling is not only the PCR method, other DNA modification procedures include transamination reaction.[10] Thus, the present strategy is expandable by choosing another interaction such as drug–protein or protein–protein. These systems will have many applications in the study of gene functions and for gene therapies that require intermittent therapeutic protein delivery.

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